



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, 15/62, C07K 14/705, A61K 38/17</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/49305</b>																																																																																																																																																																																																																																																																														
			<b>(43) International Publication Date:</b> 5 November 1998 (05.11.98)																																																																																																																																																																																																																																																																														
<b>(21) International Application Number:</b> PCT/US98/08631 <b>(22) International Filing Date:</b> 29 April 1998 (29.04.98) <b>(30) Priority Data:</b> 08/850,188 1 May 1997 (01.05.97) US <b>(71) Applicant (for all designated States except US):</b> AMGEN INC. [US/US]; Amgen Center, One Amgen Center Drive, Thousand Oaks, CA 91320-1789 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BOYLE, William, J. [US/US]; 11678 Chestnut Ridge Street, Moorpark, CA 93021 (US). WOODEN, Scott [US/US]; 1668 Sweet Briar Place, Thousand Oaks, CA 91362 (US). <b>(74) Agents:</b> ODRE, Steven, M. et al.; Amgen, Inc., Amgen Center, One Amgen Center Drive, Thousand Oaks, CA 91320-1789 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>																																																																																																																																																																																																																																																																															
<b>(54) Title:</b> CHIMERIC OPG POLYPEPTIDES																																																																																																																																																																																																																																																																																	
<b>(57) Abstract</b> <p>Chimeric polypeptides comprising fusions of an osteoprotegerin dimerization domain to a heterologous sequence are provided. Also provided are nucleic acids encoding the polypeptides, expression vectors and host cells for their production and pharmaceutical compositions comprising the polypeptides.</p>																																																																																																																																																																																																																																																																																	
<table border="0"><tr><td>TNFBp/OPG</td><td>1</td><td>MLSTVPELL LKVLLELLV QVPSGVIGL VPHLDREKA</td><td>DSVCPQKYY</td><td>50</td></tr><tr><td>TNFBp 4.0</td><td></td><td>MLSTVPELL LKVLLELLV QVPSGVIGL VPHLDREKA</td><td>DSVCPQKYY</td><td></td></tr><tr><td>TNFBp/196</td><td></td><td>MLSTVPELL LKVLLELLV QVPSGVIGL VPHLDREKA</td><td>DSVCPQKYY</td><td></td></tr><tr><td>TNFBp/217</td><td></td><td>MLSTVPELL LKVLLELLV QVPSGVIGL VPHLDREKA</td><td>DSVCPQKYY</td><td></td></tr><tr><td>TNFBp/248</td><td></td><td>MLSTVPELL LKVLLELLV QVPSGVIGL VPHLDREKA</td><td>DSVCPQKYY</td><td></td></tr><tr><td>TNFBp/304</td><td></td><td>MLSTVPELL LKVLLELLV QVPSGVIGL VPHLDREKA</td><td>DSVCPQKYY</td><td></td></tr><tr><td>TNFBp/OPG</td><td>51</td><td>HPQNSICTT KCHKOTYLN DCPQPGQDT CRECSGSFT</td><td>ASNNLRHCL</td><td>100</td></tr><tr><td>TNFBp 4.0</td><td></td><td>HPQNSICTT KCHKOTYLN DCPQPGQDT CRECSGSFT</td><td>ASNNLRHCL</td><td></td></tr><tr><td>TNFBp/196</td><td></td><td>HPQNSICTT KCHKOTYLN DCPQPGQDT 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QYRHWSENL	PQCFNCSLCL		TNFBp/196		SCSKCKKMG QVEISSCTVD RDTVCCGRN QYRHWSENL	PQCFNCSLCL		TNFBp/217		SCSKCKKMG QVEISSCTVD RDTVCCGRN QYRHWSENL	PQCFNCSLCL		TNFBp/248		SCSKCKKMG QVEISSCTVD RDTVCCGRN QYRHWSENL	PQCFNCSLCL		TNFBp/304		SCSKCKKMG QVEISSCTVD RDTVCCGRN QYRHWSENL	PQCFNCSLCL		TNFBp/OPG	151	NOTVHLSQGE KQNTVCTCHA GFFIRENECV SCSCNCKSL	CTLCLPQIE	200	TNFBp 4.0		NOTVHLSQGE KQNTVCTCHA GFFIRENECV SCSCNCKSL	CTLCLPQIE		TNFBp/196		NOTVHLSQGE KQNTVCTCHA GFFIRENECV SCSCNCKSL	CTLCLPQIE		TNFBp/217		NOTVHLSQGE KQNTVCTCHA GFFIRENECV SCSCNCKSL	CTLCLPQIE		TNFBp/248		NOTVHLSQGE KQNTVCTCHA GFFIRENECV SCSCNCKSL	CTLCLPQIE		TNFBp/304		NOTVHLSQGE KQNTVCTCHA GFFIRENECV SCSCNCKSL	CTLCLPQIE		TNFBp/OPG	201	NVKGTEDSOT TKGCGIDVTL CEEAFFFAV PTKFTFMML	VLVDNLPG	250	TNFBp 4.0		NVKGTEDSOT T...GIDVTL CEEAFFFAV PTKFTFMML	VLVDNLPG		TNFBp/196		NVKGTEDSOT T...GIDVTL CEEAFFFAV PTKFTFMML	VLVDNLPG		TNFBp/217		NVKGTEDSOT TG...GIDVTL CEEAFFFAV PTKFTFMML	VLVDNLPG		TNFBp/248		NVKGTEDSOT TG...GIDVTL CEEAFFFAV 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## CHIMERIC OPG POLYPEPTIDES

### Field of the Invention

5           The invention relates generally to chimeric polypeptides. More particularly, the invention relates to chimeric polypeptides comprising a fusion of an osteoprotegerin dimerization domain to a heterologous sequence. The polypeptides may be used in a variety of  
10   diagnostic and therapeutic applications.

### Background of the Invention

          Cells recognize a variety of signals which  
15   modulate growth, differentiation and metabolism. Effectors of cellular functions include small molecular weight organic compounds, carbohydrates, amino acids, peptides and proteins. At present, the best understood signalling process employs secretion of a signalling  
20   molecule from one cell to modulate functions of other cells (autocrine regulation). It has also been observed that secreted signalling molecules may also modulate the functions of cells which secrete them (paracrine regulation). The ability of cells to  
25   respond to external signals usually requires that the appropriate receptors which bind the signalling molecules be present on the cell surface. Protein-mediated signalling between cells involves binding of growth factors, hormones, cytokines, cell adhesion  
30   proteins and the like to cell surface receptors.

          As a class of proteins, receptors vary in their structure and mode of signal transduction. They are characterized by having an extracellular domain that is involved in binding a signalling molecule and

cytoplasmic domain which transmits an appropriate intracellular signal. Receptor expression patterns ultimately determine which cells will respond to a given ligand, while the structure of a given receptor dictates the cellular response induced by ligand binding. Receptors have been shown to transmit intracellular signals via their cytoplasmic domains by activating protein tyrosine, or protein serine/threonine phosphorylation (e.g., platelet derived growth factor receptor (PDGFR) or transforming growth factor- $\beta$  receptor-I (TGF $\beta$ R-I), by stimulating G-protein activation (e.g.,  $\beta$ -adrenergic receptor), and by modulating associations with cytoplasmic signal transducing proteins (e.g., TNFR-1 and Fas/APO) (Heldin, Cell 80, 213-223 (1995)).

The tumor necrosis factor receptor (TNFR) superfamily is a group of type I transmembrane proteins which share a conserved cysteine-rich motif which is repeated three to six times in the extracellular domain (Smith, et al. Cell 76, 953-962 (1994)). Collectively, these repeat units form the ligand binding domains of these receptors (Chen et al., Chemistry 270, 2874-2878 (1995)). The ligands for these receptors are a structurally related group of proteins homologous to TNF $\alpha$ . (Goeddel et al. Cold Spring Harbor Symp. Quart. Biol. 51, 597-609 (1986); Nagata et al. Science 267, 1449-1456 (1995)). TNF $\alpha$  binds to distinct, but closely related receptors, TNFR-1 and TNFR-2. TNF $\alpha$  produces a variety of biological responses in receptor bearing cells, including, proliferation, differentiation, and cytotoxicity and apoptosis (Beutler et al. Ann. Rev. Biochem. 57, 505-518 (1988)).

TNF $\alpha$  is believed to mediate acute and chronic inflammatory responses (Beutler et al. *ibid*). Systemic delivery of TNF $\alpha$  induces septic shock-like syndrome and

widespread tissue necrosis. Because of this, TNF $\alpha$  may be responsible for the severe morbidity and mortality associated with a variety of infectious diseases, including sepsis. Mutations in FasL, the ligand for the TNFR-related receptor Fas/APO (Suda et al. Cell 75, 1169-1178 (1993)), is associated with autoimmunity (Fisher et al. Cell 81, 935-946 (1995)), while overproduction of FasL may be implicated in drug-induced hepatitis. Thus, ligands to the various TNFR-related proteins often mediate the serious effects of many disease states, which suggests that agents that neutralize the activity of these ligands would have therapeutic value.

Soluble TNFR-1 receptors and antibodies that bind TNF $\alpha$  have been tested for their ability to neutralize systemic TNF $\alpha$  (Loetscher et al. Cancer Cells 3, 221-226 (1991)). A naturally occurring form of a secreted TNFR-1 and TNFR-2 mRNA was recently cloned, and its product tested for its ability to neutralize TNF $\alpha$  activity in vitro and in vivo (Kohno et al. Proc. Natl. Acad. Sci. USA 87, 8331-8335 (1990)). The ability of this protein to neutralize TNF $\alpha$  suggests that soluble TNF receptors function to bind and clear TNF thereby blocking the cytotoxic effects on TNFR-bearing cells.

Recombinantly-produced TNF inhibitors have also been taught in the art. For example, EP 393 438 and EP 422 339 teach the amino acid and nucleic acid sequences of a "30kDa TNF inhibitor" (also known as a p55 receptor) and a "40kDa inhibitor" (also known as a p75 receptor) as well as modified forms thereof, e.g., fragments, functional derivatives and variants. EP 393 438 and EP 422 339 also disclose methods for isolating the genes responsible for coding the

inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors. Mature recombinant 30kDa TNF inhibitor and mature recombinant 40kDa TNF inhibitor have  
5 been demonstrated to be capable of inhibiting TNF (EP 393 438, EP 422 339, PCT Publication No. WO 92/16221 and PCT Publication No. WO 95/34326).

A recently identified member of the TNFR family, termed Osteoprotegerin (OPG), is a secreted  
10 polypeptide which inhibits osteoclast maturation and markedly increases bone density in transgenic mice expressing the OPG polypeptide. OPG inhibited in vitro the formation of mature osteoclasts from hematopoietic progenitor cells and reduced the extent of bone loss in  
15 ovariectomized rats (see co-owned and co-pending U.S. Serial Nos. 08/577,788, filed December 22, 1995; 08/706,945, filed September 3, 1996; and 08/771,777 filed December 20, 1996). OPG may have benefit in the treatment of osteopenia. PCT Application No.  
20 WO96/26217 discloses a polypeptide termed Osteoclastogenesis Inhibitory Factor (OCIF) which is identical to OPG.

OPG comprises two domains having different structural and functional properties. The  
25 amino-terminal domain spanning residues 22-194 in the mature polypeptide shows homology to other members of the TNFR family, especially TNFR-2, through conservation of cysteine rich domains characteristic of TNFR family members. The carboxy terminal domain  
30 spanning residues 194-401 has no significant homology to any known sequences. Unlike a number of other TNFR family members, OPG appears to be exclusively a secreted protein and does not appear to be synthesized as a membrane associated form. Analysis of OPG by  
35 reducing and non-reducing gel electrophoresis indicated that the full-length mature polypeptide of 380 amino

acids formed a dimer having a molecular weight of about 120 kDa as compared to the monomer molecular weight of about 60 kDa. OPG polypeptides having certain truncations in the carboxy terminal domain or  
5 substitutions of certain cysteine residues within in the carboxy terminal domain formed dimeric OPG to a lesser extent and had lower biological activity compared to wild-type OPG. However, replacement of part or all of the OPG carboxy terminal domain with an  
10 Fc region of IgG restored biological activity in the OPG fusion protein to near normal levels. Based upon these observations, the amino-terminal region of OPG appeared to be required for biological activity while the carboxy-terminal domain was important for  
15 dimerization. In addition, the biological activity of OPG appeared to be enhanced when the molecule was in dimeric form.

In a therapeutic regimen, it is often desirable to modulate a biological response either by  
20 enhancing or blocking a signal received by a receptor. Enhancement of a biological response can involve increasing the affinity of the signalling molecule for a receptor, or increasing the half-life of the molecule in circulation such that it is bound to the receptor  
25 for a longer period of time. When the signalling molecule is a polypeptide, enhancement of a biological response may be achieved by constructing analogs which have amino acid sequence changes that increase binding or half-life, derivatives (e.g., polypeptides modified  
30 with water soluble polymers) to increase solubility and/or half-life, or chimeric polypeptides (e.g., polypeptides fused to the Fc region of IgG) which increase half-life, solubility and/or modify the aggregation state of the protein in circulation.  
35 Similar approaches may be taken to develop therapeutic proteins which act as antagonists by blocking a

biological response. In particular, soluble forms of transmembrane receptors which may encompass part or all of the extracellular domains have been used to prevent ligand binding and receptor activation. Soluble  
5 receptors have been developed as chemically-modified derivatives and as chimeric polypeptides.

Due to the relatively low inhibition of cytotoxicity exhibited by the 30kDa TNF inhibitor and 40kDa TNF inhibitor (Butler et al. Cytokine 6, 616-623  
10 (1994)), various groups have generated dimers of TNF inhibitor proteins (Butler et al. (1994), supra; and Martin et al. Exp. Neurol. 131, 221-228 (1995)). However, the dimers may generate an antibody response (Martin et al. (1995), supra; and Fisher et al. New  
15 Eng. J. Med., 334, 1697-1702 (1996)).

Generation of chimeric polypeptides has been described in the art. For example, construction of hybrid immunoglobulin molecules by fusion of a ligand binding partner to a human IgG chain is described in  
20 U.S. Patent Nos. 5,116,964 and 5,428,130. Construction of a chimeric polypeptide comprising the extracellular domain of a TNF receptor fused to a mouse IgG heavy chain is described in U.S. Patent No. 5,447,851. Chimeric polypeptides comprising the  
25 extracellular domain of a human PDGF receptor fused to dimerizing proteins is described in EP 0 721 983. Multimers of soluble forms of TNF receptors are described in U.S. Patent No. 5,478,925.

While fusion proteins, such as those  
30 comprising immunoglobulin constant regions, may have desirable biological properties, they can elicit an immune response which limits their usefulness as a human therapeutic.

Therefore, it is an object of the invention  
35 to provide chimeric polypeptides which enhance or block a biological response. Such polypeptides may have



increased stability, solubility, circulating half-life and decreased immunogenicity.

It is another object of the invention to provide chimeric polypeptides which combine the active region of a signalling molecule with an OPG dimerization domain wherein said chimeric polypeptides will enhance or block a biological response characteristic of the signalling molecule portion of the chimera.

It is another object of the invention to provide OPG chimeric polypeptides which form dimers, trimers and higher multimers which may have advantageous properties such as increased binding affinity, greater stability, and longer circulating half-life compared to monomeric forms.

#### Summary of the Invention

The invention provides for chimeric polypeptides comprising fusions of an OPG dimerization domain to a heterologous sequence. Also provided for are nucleic acid sequences encoding the polypeptides, expression vectors and host cells for production of the polypeptides, and pharmaceutical compositions comprising the polypeptides.

A heterologous sequence of the invention comprises an amino acid sequence of a cell signalling molecule, such as a receptor, an extracellular domain thereof, and an active fragment, derivative and analog of a receptor or an extracellular domain. In a preferred embodiment, heterologous sequences are selected from the family of TNF-like receptors. Such sequences preferentially include functional extracellular ligand binding domains and lack functional transmembrane and cytoplasmic domains. In another embodiment, the transmembrane and cytoplasmic domains are deleted in whole or in part. It is

understood that heterologous sequences of the invention do not include the amino terminal region of OPG defined by residues 22-194 as shown in U.S. Serial No.

08/577,788 filed December 22, 1995 and hereby

5 incorporated by reference, and do not include related amino acid sequences which, when fused to an OPG dimerization domain, exhibit the biological activity of OPG.

Also encompassed by the invention are  
10 multimeric polypeptides comprising covalently associated monomers of OPG chimeric polypeptides. The monomers may have identical heterologous sequences or different heterologous sequences. In a preferred embodiment, the multimeric polypeptide is a dimer,  
15 either a heterodimer (different heterologous sequences) or a homodimer (identical heterologous sequences).

The chimeric polypeptides of the invention are produced by transforming or transfecting host cells with nucleic acids encoding the polypeptide, culturing  
20 the host cells, and recovering the polypeptide from the culture. Also provided for are expression vectors and host cells for producing the chimeric polypeptides.

The chimeras are useful for detecting molecules which interact with fused heterologous  
25 sequences and thereby identifying potential new receptors and ligands. The compositions of chimeric polypeptides provided herein are useful for treatment of a variety of disorders, for example those related to receptor binding. In one embodiment, compositions  
30 comprising TNF/OPG and TNFR/OPG chimeric are used to treat TNF and TNFR mediated disorders, such as inflammation, autoimmune diseases, and disorders related to excessive apoptosis

Description of the Figures

Figure 1. Amino acid sequences of human, mouse and rat OPG dimerization domains (residues 194-401 of corresponding full-length OPG polypeptides). Conserved cysteine residues implicated in disulfide bond formation are underlined.

Figure 2. Nucleic acid and amino acid sequence of mature, full-length 30 kDa TNF inhibitor.

Figure 3. Nucleic acid and amino acid sequence of mature, full-length 40 kDa TNF inhibitor.

Figure 4. Amino acid sequences of TNFbp/OPG chimeric polypeptides. The TNFbp portion of the chimera is the full-length 30 kDa TNF inhibitor with the leader sequence (underlined) and the additional sequence VKGTEDSGTT at the carboxy terminus. OPG dimerization domains are human OPG residues 194-401, 196-401, 217-401, 248-401 and 304-401. The junction of the TNFbp and OPG sequences creates an Age I restriction site in the DNA sequence and adds a glycine codon (at position 212).

Figure 5. Gel electrophoresis analysis of TNFbp/OPG chimeric polypeptides. TNFbp/OPG chimeric plasmids were transfected into CHO d-cells. supernatants from serum-free roller bottle harvests were analyzed on a 12% polyacrylamide, Tris-glycine, non-reducing gel. Dimerization patterns were compared to a TNFbp-Fc fusion (lane 1) and TNFbp monomer (lane 8).

Figure 6. Inhibition of TNF $\alpha$  cytotoxicity on L929 cells. Serum-free conditioned medium samples of TNFbp/Fc and TNFbp/OPG [194-401] fusion polypeptides were serially diluted and assayed for inhibition of TNF $\alpha$  cytotoxicity on L929 cells.

#### Detailed Description of the Invention

The invention provides for a chimeric polypeptide comprising a fusion of an OPG dimerization domain to a heterologous sequence.

The term "heterologous sequence" refers to an amino acid sequence which is involved in cell signalling and acts to modulate cell growth, differentiation or metabolism. In general, heterologous sequences comprise extracellular ligand binding domains of cell surface receptors and their cognate ligands. When present as part of an OPG chimeric polypeptide, a heterologous sequence of the invention comprises about ten or more amino acids in length, about 20 or more amino acids in length, about 50 or more amino acids in length, and about 100 or more amino acids in length. A heterologous sequence will be of sufficient size to confer on a chimeric polypeptide a functional property such as receptor binding, enzymatic activity, inhibitor activity and the like; however, it is understood that the chimeric polypeptides will not have functional properties identical to OPG although they may share one or more functions in common with OPG. Heterologous sequences may encode full-length polypeptides or active fragments, derivatives and analogs thereof.

In preferred embodiments, chimeric OPG polypeptides include heterologous sequences encoding growth factors, cytokines, hormones, cell adhesion molecules and other polypeptide factors which are

typically secreted. Chimeric OPG polypeptides also include heterologous sequences which encode receptors for growth factors, cytokines, hormones, cell adhesion molecules, and the like, and preferably will include  
5 extracellular ligand binding domains from said receptors, and active fragments, derivatives and analogs thereof. The heterologous sequences may or may not be capable of forming dimers or higher aggregates when the sequences are present in a naturally occurring  
10 form.

The "OPG dimerization domain" refers to that portion of the OPG polypeptide which is capable of forming covalently associated multimeric polypeptides. It is understood, however, that chimeric polypeptides  
15 comprising an OPG dimerization domain are not restricted to forming dimers, but may form higher multimers as well (trimers, tetramers, etc.) The domain may have the amino acid sequence of the human osteoprotegerin dimerization domain, or it may be a  
20 fragment, derivative or analog thereof which is capable of forming covalently associated multimers. More specifically, an OPG dimerization domain will retain one or more cysteine residues which will allow formation of at least one interchain disulfide bond.  
25 In a preferred embodiment, the OPG dimerization domain has the amino acid sequence from about residues 194 to 401 inclusive of human OPG.

As used herein, the term "fragment" comprises a deletion of one or more amino acids in a heterologous  
30 sequence or in an OPG dimerization domain. The deletion may occur at the amino terminal end, the carboxy terminal end or in an internal region of the sequence. As used herein, the term "derivative" refers to a modification of the polypeptide backbone of an OPG  
35 chimera, either within the OPG dimerization domain or within the heterologous sequence. Said modifications

include, but are not limited to, attachment of water soluble polymers, hydrophobic moieties, fluorescent tags, enzymatic labels and the like. As used herein, the term "analog" refers to one or more amino acid  
5 substitutions and/or insertions within a polypeptide. Substitutions may involve conservative replacements or non-conservative replacements of amino acids which are known to one skilled in the art. Amino acid insertions may occur at the amino or carboxy terminal ends of  
10 either the OPG dimerization domain or the heterologous sequence or both, or may occur in internal regions.

#### Polypeptides

Chimeric polypeptides of the invention  
15 comprise a heterologous sequence fused at its carboxy terminus to the amino terminus an OPG dimerization domain or, alternatively, an OPG dimerization domain fused at its carboxy terminus to the amino terminus of a heterologous sequence. Chimeric polypeptides may be  
20 constructed as a direct fusion of a heterologous sequence and an OPG dimerization domain or may be constructed with a spacer or adapter region having one or more amino acids inserted between the two portions of the polypeptide. Optionally, the spacer region may  
25 encode a protease cleavage site. The precise site of the fusion is not critical and may be varied by one skilled in the art in order to optimize binding characteristics and/or biological activity of the heterologous sequence.

30 According to the invention, an OPG dimerization domain may be mammalian in origin (such as from mouse, rat or human) or may be a fragment or analog thereof which is capable of forming covalently associated dimers or higher order multimers. The amino  
35 acid sequences of rat, mouse and human OPG dimerization domains span from about residues 194-401 of their

respective full-length OPG polypeptides as shown in Figure 1 (SEQ ID NO:\_\_\_). Fragments and analogs of an OPG dimerization domain include: deletion or substitution of a cysteine residue at any of positions 195, 202, 277, 319 and 400; addition of one or more cysteine residues; rearrangement of the configuration of cysteine residues which may entail a net increase from, a net decrease from, or no change in the number of cysteine residues compared to residues 194-401 of the human OPG dimerization domain; amino-terminal truncations of OPG[194-401], e.g., 195-401, 196-401, and so forth; C-terminal truncations of OPG[194-401], e.g., 194-400, 194-399, and so forth; conservative substitutions of amino acid residues in OPG[194-401] wherein the substitutions comprise replacements with structurally or functionally similar amino acids which are known to one skilled in the art; and any combinations thereof.

Heterologous sequences which form part of a chimeric OPG polypeptide include receptors having known extracellular ligand binding domains. Examples are receptor protein-tyrosine kinases, such as the platelet-derived growth factor receptor (PDGFR) family, fibroblast growth factor receptor (FGFR) family, insulin receptor family, epidermal growth factor receptor (EGFR) family, nerve growth factor (NGFR) family, hepatocyte growth factor family (HGFR), EPH family, AXL family, TIE family, DDR family, ROR family, and other receptor protein tyrosine kinases (see van der Geer et al. Ann. Rev. Cell Biol. 10, 251-337 (1994)). Other examples of receptors having extracellular ligand binding domains include the cytokine receptor superfamily, such as G-CSF, GM-CSF ( $\alpha$  and  $\beta$  subunits), MGF, EPO, MGDF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, growth hormone,  $\alpha$ -

interferon,  $\beta$ -interferon, and  $\gamma$ -interferon receptors, the seven transmembrane domain receptor superfamily, such as acetylcholine, adrenergic, dopamine, thrombin, FSH, gonadotropin, thyrotropin, calcitonin and  
5 parathyroid hormone receptors, and cell adhesion receptors. It is understood that the receptors cited herein are merely examples and that heterologous sequences present in OPG chimeric polypeptides are not limited to the above-mentioned receptors.

10 Other heterologous sequences of the invention comprise growth factors, hormones, cytokines, cell adhesion proteins and the like. Also included are corresponding ligands for the receptor protein tyrosine kinases, ligands for cytokine receptors, ligands for  
15 seven transmembrane domain receptors, and ligands for cell adhesion receptors.

In a preferred embodiment, the heterologous sequence is a member of the TNF receptor superfamily or is derived from a member of the TNF receptor family.  
20 Members include TNFR-1, TNFR-2, TNFRp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB. Typically the extracellular domains of TNF receptors, or active fragments, derivatives and analogs thereof, are fused to an OPG dimerization domain. Active fragments of TNF  
25 receptors will have at least one cysteine rich domain, alternatively two, three or four cysteine rich domains, or alternatively one, two or three cysteine rich domains and a portion thereof, for example, two cysteine rich domains and a portion of a third domain.  
30 Activity of a TNF/OPG chimeric polypeptide may include biological activity or ligand binding activity characteristic of a TNF family member which may be evaluated using procedures known to one skilled in the art.

35 Preferred heterologous sequences comprise TNFR-1 or are derived from TNFR-1, and may be



a 30kDa TNF inhibitor, a 40 kDa TNF inhibitor, or a functionally active low molecular weight TNF inhibitor. The nucleic acid and amino acid sequence of mature, full-length 30kDa TNF inhibitor is shown in Figure 2 (SEQ ID NO:\_\_\_). The nucleic acid and amino acid sequence of mature, full-length 40kDa TNF inhibitor is shown in Figure 3 (SEQ ID NO:\_\_\_). The low molecular weight TNF inhibitors are modified forms of the 30kDa TNF inhibitor and 40 kDa TNF inhibitor which do not contain the fourth domain (amino acid residues Thr<sup>127</sup>-Thr<sup>161</sup> of the 30kDa TNF inhibitor and amino acid residues Pro<sup>141</sup>-Thr<sup>179</sup> of the 40kDa TNF inhibitor); a portion of the third domain (amino acid residues Asn<sup>111</sup>-Cys<sup>126</sup> of the 30kDa TNF inhibitor and amino acid residues Pro<sup>123</sup>-Lys<sup>140</sup> of the 40kDa TNF inhibitor); and, optionally, which do not contain a portion of the first domain (amino acid residues Asp<sup>1</sup>-Lys<sup>21</sup> of the 30kDa TNF inhibitor and amino acid residues Leu<sup>1</sup>-Lys<sup>34</sup> of the 40kDa TNF inhibitor).

The heterologous sequences of the present invention include derivatives of TNFR-1 proteins represented by the formula R<sub>1</sub>-[Cys<sup>19</sup>-Cys<sup>103</sup>]-R<sub>2</sub> and R<sub>4</sub>-[Cys<sup>32</sup>-Cys<sup>112</sup>]-R<sub>5</sub>. These proteins are deletion variants of the 30kDa TNF inhibitor and the 40kDa TNF inhibitor, respectively, and are referred to as "truncated TNFbp(s)".

By "R<sub>1</sub>-[Cys<sup>19</sup>-Cys<sup>103</sup>]-R<sub>2</sub>" is meant one or more proteins wherein [Cys<sup>19</sup>-Cys<sup>103</sup>] represents residues 19 through 103 of mature, full-length 30kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 2 (SEQ ID NO:\_\_\_) to facilitate the comparison; wherein R<sub>1</sub> represents a methionylated or nonmethionylated amine group of Cys<sup>19</sup> or of amino-terminus amino acid residue(s) selected from the group:

16

C  
 IC  
 SIC  
 NSIC (SEQ ID NO:\_\_\_)  
 NNSIC (SEQ ID NO:\_\_\_)  
 QNNSIC (SEQ ID NO:\_\_\_)  
 PQNNSIC (SEQ ID NO:\_\_\_)  
 HPQNNSIC (SEQ ID NO:\_\_\_)  
 IHPQNNSIC (SEQ ID NO:\_\_\_)  
 YIHPQNNSIC (SEQ ID NO:\_\_\_)  
 KYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 GKYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 QGKYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 PQGKYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 CPQGKYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 VCPQGKYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 SVCPQGKYIHPQNNSIC (SEQ ID NO:\_\_\_)  
 DSVCPQGKYIHPQNNSIC (SEQ ID NO:\_\_\_);

and wherein R<sub>2</sub> represents a carboxy group of Cys<sup>103</sup> or  
 of carboxy-terminal amino acid residues selected from  
 5 the group:

F  
 FC  
 FCC  
 FCCS (SEQ ID NO:\_\_\_)  
 FCCSL (SEQ ID NO:\_\_\_)  
 FCCSLC (SEQ ID NO:\_\_\_)  
 FCCSLCL (SEQ ID NO:\_\_\_);

and variants thereof.

Exemplary tumor necrosis factor binding  
 10 proteins which comprise TNFbp/OPG chimeric polypeptides

of the present invention include the following molecules: NH<sub>2</sub>-MDSVCPQGKYIHPQNNSIC-[Cys<sup>19</sup>-Cys<sup>103</sup>]-FC-COOH (also referred to as 30kDa TNFbp 2.6C105); NH<sub>2</sub>-MDSVCPQGKYIHPQNNSIC-[Cys<sup>19</sup>-Cys<sup>103</sup>]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.6C106); NH<sub>2</sub>-MDSVCPQGKYIHPQNNSIC-[Cys<sup>19</sup>-Cys<sup>103</sup>]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.6N105); NH<sub>2</sub>-MYIHPQNNSIC-[Cys<sup>19</sup>-Cys<sup>103</sup>]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d8); NH<sub>2</sub>-M-[Cys<sup>19</sup>-Cys<sup>103</sup>]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d18); and NH<sub>2</sub>-MSIS-[Cys<sup>19</sup>-Cys<sup>103</sup>]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d15), either methionylated or nonmethionylated, and variants and derivatives thereof.

By "R<sub>4</sub>-[Cys<sup>32</sup>-Cys<sup>112</sup>]-R<sub>5</sub>" is meant one or more proteins wherein [Cys<sup>32</sup>-Cys<sup>112</sup>] represents residues Cys<sup>32</sup> through Cys<sup>112</sup> of mature, full-length 40kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 3 (SEQ ID NO:\_\_) to facilitate the comparison; wherein R<sub>4</sub> represents a methionylated or nonmethionylated amine group of Cys<sup>32</sup> or of amino-terminus amino acid residue(s) selected from the group:

C	
MC	
QMC	
AQMC	(SEQ ID NO:__)
TAQMC	(SEQ ID NO:__)
QTAQMC	(SEQ ID NO:__)
DQTAQMC	(SEQ ID NO:__)
YDQTAQMC	(SEQ ID NO:__)
YYDQTAQMC	(SEQ ID NO:__)
EYYDQTAQMC	(SEQ ID NO:__)
REYYDQTAQMC	(SEQ ID NO:__)
LREYYDQTAQMC	(SEQ ID NO:__)

RLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 CRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 TCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 STCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 GSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 PGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 EPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 PEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 APEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 YAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 PYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 TPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 FTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 AFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 VAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 QVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 AQVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 PAQVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_)  
 LPAQVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:\_\_\_);

and wherein R<sub>5</sub> represents a carboxy group of Cys<sup>112</sup> or of carboxy-terminal amino acid residues selected from the group:

5

R  
 RL  
 RLC  
 RLCA (SEQ ID NO:\_\_\_)  
 RLCAP (SEQ ID NO:\_\_\_)  
 RLCAPL (SEQ ID NO:\_\_\_)  
 RLCAPLR (SEQ ID NO:\_\_\_)  
 RLCAPLRK (SEQ ID NO:\_\_\_)  
 RLCAPLRKC (SEQ ID NO:\_\_\_)  
 RLCAPLRKCR (SEQ ID NO:\_\_\_)

and variants thereof.

As shown in Example 1, a hybrid DNA molecule encoding TNFbp 4.0, the full-length 30 kDa TNF inhibitor (Figure 2) with the additional sequence VKGTEDSGTT extending from the carboxy terminus, and  
5 human OPG [194-401] was constructed. The resulting chimeric polypeptide, termed TNFbp/OPG[194-401] has the amino acid sequence as shown in Figure 4. Upon expression, the mature chimeric polypeptides formed dimers in conditioned medium of transfected host cells  
10 as determined by non-reducing SDS-PAGE (see Figure 5). Additional TNFbp fusions were constructed to amino terminal truncations of the human OPG dimerization domain. These constructs are designated TNFbp/OPG[196-401], TNFbp/OPG[217-401], TNFbp/OPG[248-401], and  
15 TNFbp/OPG[304-401] and the amino acid sequences are shown in Figure 4. OPG[194-401] has the full complement of five cysteine residues which are involved in covalent association of OPG dimerization domains. OPG[196-401] lacks one cysteine residue at position  
20 195, OPG[217-401] and OPG[248-401] lacks a second cysteine residue at position 202, and OPG[304-401] lacks a third cysteine residue at position 277 (see Figure 1 for location of cysteine residues). The chimeric polypeptides produced in conditioned medium of  
25 transfected CHO<sup>d</sup>- host cells were analyzed by non-reducing SDS-PAGE (Figure 5). In the L929 cytotoxicity assay, the TNFbp/OPG[194-401] chimera showed activity similar to a TNFbp/Fc chimera (Figure 6).

30 The invention also provides for chimeric OPG polypeptides which form multimers (i.e., dimers, trimers and higher multimers). Multimers of the invention comprise covalently associated monomeric OPG chimeras wherein the monomers may have identical  
35 heterologous sequence or different heterologous sequences. Preferably, the chimeric polypeptides are

dimers or trimers. Preparations of multimeric polypeptides will be essentially free of monomeric OPG chimeras which are not covalently associated and of inactive multimers. Such preparations are made using techniques available to one skilled in the art

Modifications of chimeric OPG polypeptides are encompassed by the invention and include post-translational modifications (e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of OPG which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1kDa and about 100kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules

will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release  
5 desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

10 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the  
15 art, e.g. EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al. Exp. Hematol. 20, 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through  
20 amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the  
25 N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol  
30 molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire N-terminally chemically modified protein. Using polyethylene  
35 glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol

molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemically modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The chimeric OPG polypeptides of the invention are isolated and purified from other constituents present in lysates or supernatants of host cells expressing the polypeptides. In one embodiment, the polypeptide is free from association with other human proteins, such as the expression product of a bacterial host cell. Also provided by the invention is a method for the purification of OPG chimeric polypeptides. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-OPG antibody or biotin-streptavidin affinity complex and the like. When preparations of selected multimeric OPG chimeras are desired, the purification



method may be carried out to separate species of different aggregation states, for example, separation of monomeric from dimeric OPG chimeras, or separation of dimeric from tetrameric OPG chimeras.

5           Chimeric OPG polypeptides may be used in assays to screen for binding molecules. Examples of such molecules include, but are not limited to, nucleic acids, polypeptides, small molecular weight peptides, carbohydrates, lipids and small molecular weight  
10 organic compounds. Assays will employ combining candidate molecules (either purified or unpurified) with chimeric OPG polypeptides under conditions that allowing binding, and measuring the extent of binding to the chimeric polypeptide. Binding measurements are  
15 made using detection systems available to one skilled in the art, such as radioactivity, enzymatic activity, fluorescence, and surface plasmon resonance.

#### Nucleic Acids

20           The invention provides for an isolated nucleic acid encoding a chimeric polypeptide having an OPG dimerization domain fused to a heterologous sequence. The nucleic acids encode a chimeric OPG polypeptide wherein the heterologous sequence is a cell  
25 signalling molecule such as a receptor or a receptor ligand. In a preferred embodiment, the heterologous nucleic acid sequence encodes a polypeptide of the TNFR family, or a fragment, derivative or analog thereof, provided however that the heterologous nucleic acid  
30 sequence does not encode OPG[22-194] as shown in U.S. Serial No. 08/577,788 filed December 22, 1995, or a homologous sequence which, when fused to an OPG dimerization domain, has the biological activity of OPG.

35           The nucleic acids of the invention encode chimeric OPG polypeptides selected from the following:

a) the nucleic acid sequences which encode the polypeptides shown in Figure 1 (SEQ ID NO: \_\_\_\_ ) or complementary strands thereof; and

5 b) the nucleic acids sequences which hybridize under high stringency conditions with the sequences in (a), and degenerate sequences thereof, provided however that the polypeptides do not have the biological activity of OPG. Nucleic acids encoding OPG chimeric polypeptides may hybridize over part or all of  
10 the nucleic acid sequences encoding the OPG dimerization domains shown in Figure 1 (SEQ ID NO: \_\_\_\_ ).

The conditions for hybridization are generally of high stringency using temperatures,  
15 solvents and salt concentrations wherein the hybridizing sequences are about 12-20°C below the melting temperature ( $T_m$ ) of the perfectly matched duplex. Equivalent stringency to these conditions may be readily ascertained by one skilled in the art by  
20 adjusting salt and organic solvent concentrations and temperature. Specific hybridization conditions are described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

25 Preferred sequences include nucleic acids which encode chimeric OPG polypeptides having rat, mouse and human OPG dimerization domains. DNA encoding human OPG dimerization domain was provided in a full-length human OPG plasmid designated pRcCMV - human OPG  
30 and deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under accession no. 69969. DNA encoding rat OPG dimerization domain was provided in a full-length rat OPG plasmid designated pMOB-B1.1 and deposited with the American  
35 Type Culture Collection, Rockville, MD on December 27,

1995 under ATCC accession no. 69970. DNA encoding mouse OPG dimerization domain was provided in a full-length mouse OPG plasmid designated pRcCMV-murine OPG and deposited with the American Type Culture  
5 Collection, Rockville, MD on December 27, 1995 under accession no. 69971. The nucleic acids of the invention will hybridize under stringent conditions to the DNA inserts of ATCC accession nos. 69969, 69970, and 69971.

10 In a preferred embodiment, heterologous sequences will comprise nucleic acids encoding TNFR-1, and fragments, derivatives and analogs thereof, such as the TNF 30kDa inhibitor or TNF 40kDa inhibitor. Presently preferred heterologous sequences include  
15 those nucleic acids encoding 30kDa TNFbp 2.6C105, 30kDa TNFbp 2.6C106, 30kDa TNFbp 2.6N105, 30kDa TNFbp 2.3d8, 30kDa TNFbp 2.3d18 and 30kDa TNFbp 2.3d15.

Also provided by the invention are nucleic acids encoding variants of an OPG chimeric polypeptide  
20 wherein the variations may be in the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives comprise addition, substitution, insertion or deletion of one or more nucleotides such that the resulting sequences encode  
25 chimeric OPG polypeptides comprising one or more amino acid residues which have been added, deleted, inserted or substituted in either the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives may be naturally occurring, such as by  
30 splice variation or polymorphism, or may be constructed using site-directed mutagenesis techniques available to the skilled worker. Chimeric OPG polypeptide variants are described in the previous section entitled  
"Polypeptides" and it is anticipated that nucleic acids  
35 encoding all variants disclosed therein, and degenerate molecules thereof, are encompassed by the invention.

Examples of the nucleic acids of the invention include cDNA, genomic DNA, synthetic DNA and RNA. cDNA is obtained from libraries prepared from mRNA isolated from various tissues expressing OPG. In humans, tissue sources for OPG include kidney, liver, placenta and heart. Genomic DNA encoding OPG is obtained from genomic libraries which are commercially available from a variety of species. Synthetic DNA is obtained by chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding region and flanking sequences (see U.S. Patent No. 4,695,623). RNA may be obtained in large quantities use of procaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and RNA polymerase.

Nucleic acid sequences of the invention are useful for the expression of chimeric OPG polypeptides. Expression may be carried out in transfected host cells for production of recombinant protein in quantities sufficient for diagnostic or therapeutic applications. In addition, chimeric OPG polypeptides may be expressed in vivo and secreted into the circulation to provide therapeutic benefit.

#### Vectors and Host Cells

Expression vectors containing nucleic acid sequences encoding OPG fusion proteins, host cells transformed with said vectors and methods for the production of OPG fusion proteins are also provided by the invention. An overview of expression of recombinant proteins is found in Methods of Enzymology v. 185, Goeddel, D.V. ed. Academic Press (1990).

Host cells for the production of OPG fusion proteins include procaryotic host cells, such as E. coli, yeast, plant, insect and mammalian host cells.

E. coli strains such as HB101 or JM101 are suitable for expression. Preferred mammalian host cells include COS, CHOd-, 293, CV-1, 3T3, baby hamster kidney (BHK) cells and others. Mammalian host cells are preferred  
5 when post-translational modifications, such as glycosylation and polypeptide processing, are important for OPG chimera activity. Mammalian expression allows for the production of secreted polypeptides which may be recovered from the growth medium.

10 Vectors for the expression of OPG chimeric polypeptides contain at a minimum sequences required for vector propagation and for expression of the cloned insert. These sequences include a replication origin, selection marker, promoter, ribosome binding site,  
15 enhancer sequences, RNA splice sites and transcription termination site. Vectors suitable for expression in the aforementioned host cells are readily available and the nucleic acids of the invention are inserted into the vectors using standard recombinant DNA techniques.  
20 Vectors for tissue-specific expression of OPG chimeric polypeptides are also included. Such vectors include promoters which function specifically in liver, kidney or other organs for production in mice, and viral vectors for the expression of OPG in targeted human  
25 cells.

Using an appropriate host-vector system, OPG chimeric polypeptides are produced recombinantly by culturing a host cell transformed with an expression vector containing nucleic acid sequences encoding an  
30 OPG chimeric polypeptide under conditions such that the polypeptide is produced, and isolating the product of expression. OPG chimeras are produced in the supernatant of transfected mammalian cells or in inclusion bodies of transformed bacterial host cells.  
35 OPG chimeras so produced may be purified by procedures known to one skilled in the art as described below.

Expression vectors for mammalian hosts are exemplified by plasmids such as pDSR $\alpha$  described in PCT Application No. 90/14363; see also Methods in Enzymology vol. 185, D.V. Goeddel, ed. pp. 487-511 for additional examples.

5 A variety of expression vectors are available for bacterial host cells and are described in Methods in Enzymology, ibid. pp. 14-37 and references cited therein. It is anticipated that the specific plasmids and host cells described are for illustrative purposes  
10 and that the choice of any specific plasmid and host cell for expression of an OPG chimeric polypeptide will depend upon consideration of a variety of factors by one skilled in the art.

#### 15 Antibodies

Also encompassed by the invention are antibodies specifically binding to an OPG chimeric polypeptide. Antigens for the generation of antibodies may be full-length polypeptides or peptides spanning a  
20 portion of the OPG sequence. Immunological procedures for the generation of polyclonal or monoclonal antibodies reactive with OPG are known to one skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor  
25 Laboratory Press, Cold Spring Harbor N.Y. (1988)). Antibodies so produced are characterized for binding specificity and epitope recognition using standard enzyme-linked immunosorbent assays. Antibodies also include chimeric antibodies having variable and  
30 constant domain regions derived from different species. In one embodiment, the chimeric antibodies are humanized antibodies having murine variable domains and human constant domains. Also encompassed are complementary determining regions grafted to a human  
35 framework (so-called CDR-grafted antibodies). Chimeric

and CDR-grafted antibodies are made by recombinant methods known to one skilled in the art. Also encompassed are human antibodies made in mice.

Anti-OPG chimera antibodies of the invention  
5 may be used as an affinity reagent to purify OPG from biological samples. In one method, the antibody is immobilized on CNBr-activated Sepharose and a column of antibody-Sepharose conjugate is used to remove OPG from liquid samples. Antibodies are also used as diagnostic  
10 reagents to detect and quantitate OPG in biological samples by methods described below.

#### Pharmaceutical compositions

The invention also provides for  
15 pharmaceutical compositions comprising a therapeutically effective amount of an OPG chimeric polypeptide together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The term "therapeutically effective  
20 amount" refers to an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values  
25 and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascorbic acid or sodium metabisulfite. Also encompassed are  
30 compositions comprising OPG chimeric polypeptides modified with water soluble polymers to increase solubility or stability. Compositions may also comprise incorporation of OPG chimeric polypeptides into liposomes, microemulsions, micelles or vesicles  
35 for controlled delivery over an extended period of time. Selection of a particular composition will

depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of components suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

Pharmaceutical compositions of chimeric OPG polypeptides are useful for treatment of receptor-mediated disorders, for example disorders resulting from the function (or lack thereof) of protein tyrosine kinases, cytokine, seven transmembrane domain, and cell adhesion receptors. Disorders resulting from the function (or lack thereof) of the corresponding polypeptide ligands of the above referenced receptors may also be treated. In one embodiment, compositions comprising TNF/OPG chimeras are used to treat TNF-related disorders such as inflammation, autoimmune diseases and conditions marked by excessive apoptosis. Chimeras of the invention may act as agonists to stimulate receptor activation and associated changes in cell activity, or chimeras may be antagonists which block receptor function.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be



suitable for delivery to cells and tissues as part of an anti-sense or gene therapy regimen.

The following examples are offered to more fully illustrate the invention, but are not construed  
5 as limiting the scope thereof.

#### EXAMPLE 1

##### Construction and Expression of TNFbp/OPG fusion proteins

10

The TNFbp/OPG[196-401] chimeric gene was prepared in a two step PCR process. A first round of PCR was designed to produce overlapping PCR products from each gene. The templates used were plasmids  
15 p2302, containing the gene encoding TNFbp 4.0 (Figure 4) fused to the Fc region of human IgG1, and plasmid pRcCMV-human OPG (ATCC accession no. 69969), containing the gene for human OPG. The PCR products were gel purified and used as a template to create the chimeric  
20 gene. Primers used for the PCR reactions are as follows: 1275-51 (containing a 5' XbaI site, consensus Kozak and the start of the hTNFbp gene) and 1368-82 (containing a portion of OPG cDNA, an AgeI site and the 3' end of the human TNFbp 4.0 sequence) were used to  
25 amplify the TNFbp gene from p2302; 1368-83 (containing the 3' end of TNFbp, an AgeI site and the 5' end of the hOPG C-terminal domain) and 1295-27 (containing a SalI site and the 3' end of the OPG cDNA) were used to amplify the OPG[196-401] gene from pRcCMV-human OPG. A  
30 second PCR reaction used primers 1275-51 and 1295-27 to generate the chimeric gene.

The PCR product was cut with XbaI/SalI and subcloned into the pDSR $\alpha$ 2 expression vector to give plasmid p389-1. The expression cassette contains a  
35 SV40 early promoter driving the expression of the chimeric gene and also includes an SV40 late intron, an

HTLV translation enhancing signal and an  $\alpha 2$ -FSH polyadenylation signal (DeClerck, et al. J. Biol. Chem. 266, 3893-3899 (1991)). The pDSR $\alpha 2$  vector also contains a DHFR cassette for selection in CHO d- cells.

5

#### Primer Sequences:

1275-51:

(SEQ ID NO:\_\_\_)

10 5'-CGC TCTAGA CCACC ATG GGC CTC TCC ACC GTG-3'  
XbaI Kozak M G L S T V

1368-82:

(SEQ ID NO:\_\_\_)

15 5'-ACACAGGGTAACATCTAT ACCGGT GGTGCCTGAGTCCTCAG-3'  
hOPG C-terminus AgeI hTNFbp

1368-83:

(SEQ ID NO:\_\_\_)

20 5'-CTGAGGACTCAGGCACC ACCGGT ATAGATGTTACCCTGTG-3'  
E D S G T T G I D V T L  
TNFbp AgeI hOPG C-terminus

1295-27:

25 (SEQ ID NO:\_\_\_)

5'-CCTCT GTCGAC TA TTA TAA GCA GCTTATTTTCACGGATTG-3'  
SalI \* \* L C.... OPG-->

30 Other constructs with truncated OPG dimerization doamins were created as follows:

The primer pair for OPG[194-401] was 1295-27 and 1428-89.

1428-89:

(SEQ ID NO:\_\_\_)

TCA ACCGGT AAA TGT GGA ATA GAT GTT AC

5           AgeI    K    C    G    I    D    V    T

The primer pair for OPG[217-401] was 1295-27 and 1388-50.

10 1388-50:

(SEQ ID NO:\_\_\_)

GTTT ACCGGT CCT AAC TGG CTT AGT GTC

          AgeI    P    N    W    L    S    V

15 The primer pair for OPG[248-401] was 1295-27 and 1388-51.

1388-51:

(SEQ ID NO:\_\_\_)

20 AGC ACCGGT GAA CAG ACT TTC CAG CTG

          AgeI    E    Q    T    F    Q    L

The primer pair for OPG[304-401] was 1295-27 and 1388-52.

25

1388-52:

(SEQ ID NO:\_\_\_)

GGAA ACCGGT CCG GGA AAG AAA GTG GG

          AgeI    P    G    K    K    V    G

30

The corresponding TNFbp/OPG fusion was constructed by excising the AgeI/SalI OPG fragment from p389-1 and replacing it with AgeI/SalI digested OPG PCR products from the above reactions. The amino acid sequences encoded by the above TNFbp/OPG constructs are shown in Figure 4.

35

Transient transfections were performed in COS-7 cells by electroporation. Ten  $\mu\text{g}$  of plasmid DNA was electroporated into  $2 \times 10^6$  cells in 0.8 mls of DMEM. The electroporations were done in 0.4 cm cuvettes at 1.6 kV, 25 mF and 200 ohms. The electroporated cells were plated in 10-cm dishes in DMEM containing 10% FBS, 1x glutamine/penicillin/streptomycin, 1x non-essential amino acids, 1x Na-pyruvate. The following day the media was changed to media containing only 1% FBS. After an additional 72 hours, the conditioned media was harvested and 17  $\mu\text{l}$  was electrophoresed on a 12% denaturing, non-reducing gel. These gels were blotted and analyzed by western blots for the presence of monomer and covalently-linked dimers. The primary antibody was anti-TNFbp (R&D systems, AB-225-PB) at a 1:1000 dilution and the secondary antibody was HRP, rabbit anti-goat (Pierce) at a 1:1000 dilution.

Stable transfections were done in CHO d-cells by calcium phosphate precipitation (DeClerck et al., supra). The transfection was performed as described except that 20  $\mu\text{g}$  of PvuI linearized plasmid was used with 10  $\mu\text{g}$  of herring sperm carrier DNA and 10  $\mu\text{l}$  of calcium phosphate maximizer (Clontech) to transfect to a 10-cm dish containing approximately  $5 \times 10^5$  cells. After 2 weeks in HT- selection, colonies were ring-cloned and expanded into 24-well plates. Once confluent, two day serum-free conditioned media (SFCM) was prepared and analyzed for the expression of TNFbp/OPG fusion protein by western blot. High expressing clones were expanded and grown in roller bottles for 7d SFCM harvests. The results are shown in Figure 5.

## EXAMPLE 2

## Biological Activity of TNFbp/OPG chimeric proteins

WEHI Cytotoxicity Assay

5           The WEHI assay is an in vitro cell proliferation assay (Edwards et al. Endocrinology 128,989-996 (1991)). The cell lines are sensitive to TNF- $\alpha$  (i.e., TNF- $\alpha$  is cytotoxic). In the presence of a TNF- $\alpha$  inhibitor, the cells were protected from the  
10 cytotoxic effect and thus were able to proliferate.

          TNF-sensitive WEHI 164 clone 13 cells are suspended at a concentration of  $20 \times 10^4$  cells/ml in RPMI (Gibco, Grand Island, NY) medium supplemented with 5% Fetal Calf Serum (Hyclone) and penicillin  
15 50U/ml:streptomycin 50 mg/ml. One hundred microliters of this cell suspension are placed in each well of flat-bottomed 96-cell microtiter plates, and the cells are allowed to adhere for 4-6 hours at 37°C in 7% CO<sub>2</sub>. Medium is then aspirated, and 0.60 mg/ml actinomycin-D  
20 (Sigma Chemical Co., St. Louis, MO) is added to each well. A standard curve using serial dilutions at 0, 0.001 0.01, 0.1, 1, 10, 100 U/ml recombinant human TNF is run with each assay. Serially diluted 10-fold concentrations of TNFbp/OPG chimeras from serum-free  
25 conditioned medium are further diluted in RPMI-1640 medium containing 5% FBS and then added to duplicate wells (50  $\mu$ l/well) containing adherent WEHI 164 cells after the addition of recombinant mouse TNF- $\alpha$ . WEHI-164 clone 13 cells are incubated for 18 hours at 37°C  
30 in 5% CO<sub>2</sub>. Maximal killing is determined by adding 0.02% Triton X-100 (TX-100) to test wells. After incubation, 70  $\mu$ l medium are aspirated, and 50  $\mu$ l of a 1 mg/mL solution of the organic dye MTT tetrazolium (3-[4,5-dimethylthiozol-2-yl]2,5-diphenyl tetrazolium bromide; Sigma) is added, and cells are incubated for  
35

an additional 4-6 hours. All supernatants are then removed, and 50  $\mu$ l DMF/SDS solution (20% SDS, and 50% N,N dimethylformamide, pH 4.7) is added to each well. The DMF/SDS solution is pipetted up and down several times until all MTT crystals are dissolved, and cells were incubated for an additional 2-22 hours. The absorbances (abs) are read on a Vmax reader at 570-650. The percent specific cytotoxicity is calculated from optical densities using the formula: % specific cytotoxicity = 100% X [abs(cells + medium) - abs(cells + sample)]/abs(cells + medium) - abs(cells + TX-100)]. The number of units of TNF in each sample is determined using the percent specific cytotoxicities of the murine standards.

#### L929 Cytotoxicity Assay

The L929 cytotoxicity assay is an in vitro cell proliferation assay (Parmely et al. J. Immunol. 151, 389-396 (1993), the disclosure of which is hereby incorporated by reference) which also assesses the cytotoxicity of TNF- $\alpha$ -sensitive killing. The cell lines are sensitive to TNF- $\alpha$  (i.e.; TNF- $\alpha$  is cytotoxic). In the presence of a TNF- $\alpha$  inhibitor, the cells are protected from the cytotoxic effect and thus survive and are able to proliferate.

The L929 cell line was obtained from the American Type Culture Collection (catalog number ATCC CCL 1 NCTC clone 929), as described previously by Parmely et. al. (1993), supra. L929 cells were grown in tissue culture flasks in Dulbecco's MEM with 10 % fetal calf serum (FCS) to 80 % confluence. Cells were trypsinized and seeded at 8,000-10,000 cells/well in 100 ml into Falcon #3072 96 well plates and incubated for 20 to 40 hours at 37 °C in 5% CO<sub>2</sub>. Samples of TNFbp/Fc or TNFbp/OPG [194-401] polypeptides were

serially diluted in medium and added in triplicate followed by addition of TNF $\alpha$  to reach a final concentration of 0.5 mg/ml. The cultures were incubated at 37 °C overnight and cell density was measured by crystal violet. Medium was removed by inverting the 96 well plates. Cells were fixed in 100  $\mu$ l 100% methanol for 2 minutes. After removal of methanol the plates were allowed to dry for 10 minutes. 100  $\mu$ l of 0.10% crystal violet stain in 20% methanol was added and plates were stained for 10 minutes at room temperature. Excess stain was removed by inverting plates. Plates were washed by dipping three times in ice-cold distilled water and excess water was removed from the wells by gently blotting plates on a tissue. 100  $\mu$ l of 100% methanol was added to stained cells and optical density was measured at 595 nm. Media control reactions contained L929 cells and medium alone, and TNF control reactions contained L929 cells with 0.5 ng/ml TNF $\alpha$ .

The activity in this assay of TNFbp/OPG fusions constructed as described in Example 1 is shown in Figure 6.

\* \* \*

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

(i) APPLICANT: Amgen Inc.

10

(ii) TITLE OF INVENTION: CHIMERIC OPG POLYPEPTIDES

(iii) NUMBER OF SEQUENCES: 87

(iv) CORRESPONDENCE ADDRESS:

15

(A) ADDRESSEE: Amgen Inc.

(B) STREET: 1840 Dehavilland Drive

(C) CITY: Thousand Oaks

(D) STATE: California

(E) COUNTRY: USA

20

(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

25

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

30

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

35

(A) NAME: Winter, Robert B.

(C) REFERENCE/DOCKET NUMBER: A-452

## (2) INFORMATION FOR SEQ ID NO:1:

40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: protein

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asn Ser Ile Cys

1

55

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

60

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein



5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
Asn Asn Ser Ile Cys  
1 5

10 (2) INFORMATION FOR SEQ ID NO:3:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
15 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
Gln Asn Asn Ser Ile Cys  
25 1 5

(2) INFORMATION FOR SEQ ID NO:4:  
30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
Pro Gln Asn Asn Ser Ile Cys  
45 1 5

(2) INFORMATION FOR SEQ ID NO:5:  
(i) SEQUENCE CHARACTERISTICS:  
50 (A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  
His Pro Gln Asn Asn Ser Ile Cys  
60 1 5

40

## (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5

## (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:  
Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10

10 (2) INFORMATION FOR SEQ ID NO:10:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
15 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:  
30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:  
Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10

45 (2) INFORMATION FOR SEQ ID NO:12:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
50 (B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10 15

42

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

15

Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys  
1 5 10 15

## 20 (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

35 Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile  
1 5 10 15  
Cys

40

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

55

Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser  
1 5 10 15

Ile Cys

60

## (2) INFORMATION FOR SEQ ID NO:16:

43

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 10
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
- 15 Phe Cys Cys Ser  
1
- (2) INFORMATION FOR SEQ ID NO:17:
- 20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: protein
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
- Phe Cys Cys Ser Leu  
1 5
- 35 (2) INFORMATION FOR SEQ ID NO:18:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
40 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 45
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
- 50 Phe Cys Cys Ser Leu Cys  
1 5
- (2) INFORMATION FOR SEQ ID NO:19:
- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
60 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5       Phe Cys Cys Ser Leu Cys Leu  
      1                   5

(2) INFORMATION FOR SEQ ID NO:20:

10       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 4 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

15       (ii) MOLECULE TYPE: protein

20       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

      Ala Gln Met Cys  
      1

25       (2) INFORMATION FOR SEQ ID NO:21:

      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 5 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

35       (ii) MOLECULE TYPE: protein

40       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

      Thr Ala Gln Met Cys  
      1                   5

45       (2) INFORMATION FOR SEQ ID NO:22:

      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 6 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

50       (ii) MOLECULE TYPE: protein

55       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

60       Gln Thr Ala Gln Met Cys  
      1                   5

(2) INFORMATION FOR SEQ ID NO:23:

45

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
- 15 Asp Gln Thr Ala Gln Met Cys  
1 5
- (2) INFORMATION FOR SEQ ID NO:24:
- 20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: protein
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
- Tyr Asp Gln Thr Ala Gln Met Cys  
1 5
- 35 (2) INFORMATION FOR SEQ ID NO:25:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
40 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
- 50 Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
1 5
- (2) INFORMATION FOR SEQ ID NO:26:
- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
60 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

5      Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
      1                    5                    10

(2) INFORMATION FOR SEQ ID NO:27:

10      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 11 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

15      (ii) MOLECULE TYPE: protein

20      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

      Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
      1                    5                    10

25      (2) INFORMATION FOR SEQ ID NO:28:

      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 12 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

35      (ii) MOLECULE TYPE: protein

40      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

      Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
      1                    5                    10

45      (2) INFORMATION FOR SEQ ID NO:29:

      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 13 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

50      (ii) MOLECULE TYPE: protein

55      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

60      Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
      1                    5                    10

(2) INFORMATION FOR SEQ ID NO:30:



47

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 14 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
5      (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

15      Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
        1                      5                      10

(2) INFORMATION FOR SEQ ID NO:31:

20      (i) SEQUENCE CHARACTERISTICS:  
        (A) LENGTH: 15 amino acids  
        (B) TYPE: amino acid  
        (C) STRANDEDNESS: single  
        (D) TOPOLOGY: linear

25      (ii) MOLECULE TYPE: protein

30      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

        Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
        1                      5                      10                      15

35      (2) INFORMATION FOR SEQ ID NO:32:

        (i) SEQUENCE CHARACTERISTICS:  
            (A) LENGTH: 16 amino acids  
            (B) TYPE: amino acid  
40              (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

45

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

50      Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
        1                      5                      10                      15

55      (2) INFORMATION FOR SEQ ID NO:33:

        (i) SEQUENCE CHARACTERISTICS:  
            (A) LENGTH: 17 amino acids  
            (B) TYPE: amino acid  
60              (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:  
Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met  
1 5 10 15  
Cys  
10
- (2) INFORMATION FOR SEQ ID NO:34:
- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:  
Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln  
1 5 10 15  
Met Cys  
30
- (2) INFORMATION FOR SEQ ID NO:35:
- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:  
Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala  
1 5 10 15  
Gln Met Cys  
50
- (2) INFORMATION FOR SEQ ID NO:36:
- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
60 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr  
1 5 10 15  
Ala Gln Met Cys  
20

10 (2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:  
15 (A) LENGTH: 21 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln  
1 5 10 15  
Thr Ala Gln Met Cys  
20

(2) INFORMATION FOR SEQ ID NO:38:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp  
1 5 10 15  
Gln Thr Ala Gln Met Cys  
20

55 (2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr  
1 5 10 15  
Asp Gln Thr Ala Gln Met Cys  
20

(2) INFORMATION FOR SEQ ID NO:40:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr  
1 5 10 15  
Tyr Asp Gln Thr Ala Gln Met Cys  
20

(2) INFORMATION FOR SEQ ID NO:41:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu  
1 5 10 15  
Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
20 25

(2) INFORMATION FOR SEQ ID NO:42:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
- Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg  
1 5 10 15
- 10 Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
20 25
- (2) INFORMATION FOR SEQ ID NO:43:
- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
- Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu  
1 5 10 15
- 30 Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
20 25
- (2) INFORMATION FOR SEQ ID NO:44:
- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
- Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg  
1 5 10 15
- 50 Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
20 25
- (2) INFORMATION FOR SEQ ID NO:45:
- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
- Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys  
 1 5 10 15
- Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
 20 25
- 10 (2) INFORMATION FOR SEQ ID NO:46:
- (i) SEQUENCE CHARACTERISTICS:  
 15 (A) LENGTH: 30 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
- Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr  
 1 5 10 15
- 30 Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
 20 25 30
- (2) INFORMATION FOR SEQ ID NO:47:
- 35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 31 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
- Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser  
 1 5 10 15
- 50 Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys  
 20 25 30
- (2) INFORMATION FOR SEQ ID NO:48:
- 55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 4 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 60 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Arg Leu Cys Ala  
1

10 (2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

25 Arg Leu Cys Ala Pro  
1 5

(2) INFORMATION FOR SEQ ID NO:50:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Leu Cys Ala Pro Leu  
1 5

45 (2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
50 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

60 Arg Leu Cys Ala Pro Leu Arg  
1 5

(2) INFORMATION FOR SEQ ID NO:52:

54

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:
- 15 Arg Leu Cys Ala Pro Leu Arg Lys  
1 5
- (2) INFORMATION FOR SEQ ID NO:53:
- 20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
25 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
- Arg Leu Cys Ala Pro Leu Arg Lys Cys  
1 5
- 35 (2) INFORMATION FOR SEQ ID NO:54:
- (i) SEQUENCE CHARACTERISTICS:  
40 (A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:
- 50 Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg  
1 5 10
- 55 (2) INFORMATION FOR SEQ ID NO:55:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
60 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA



55

(ix) FEATURE:  
    (A) NAME/KEY: CDS  
    (B) LOCATION: 15..32

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

10 CGCTCTAGAC CACC ATG GGC CTC TCC ACC GTG 32  
    Met Gly Leu Ser Thr Val  
        1                    5

(2) INFORMATION FOR SEQ ID NO:56:

15 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 6 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

25 Met Gly Leu Ser Thr Val  
    1                    5

(2) INFORMATION FOR SEQ ID NO:57:

30 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 41 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACACAGGGTA ACATCTATAC CGGTGGTGCC TGAGTCCTCA G 41

45 (2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 40 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

55 (ix) FEATURE:  
    (A) NAME/KEY: CDS  
    (B) LOCATION: 3..40

60

56

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CT GAG GAC TCA GGC ACC ACC GGT ATA GAT GTT ACC CTG TG  
 Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu  
 1 5 10

40

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu  
 1 5 10

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 40 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCTCTGTCGA CTATTATAAG CAGCTTATTT TCACGGATTG

40

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 10..29

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TCAACCGGT AAA TGT GGA ATA GAT GTT AC  
 Lys Cys Gly Ile Asp Val  
 1 5

29

60

57

## (2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Cys Gly Ile Asp Val  
1 5

## (2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 11..28

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GTTTACCGGT CCT AAC TGG CTT AGT GTC  
Pro Asn Trp Leu Ser Val  
1 5

28

## (2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Pro Asn Trp Leu Ser Val  
1 5

## (2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

58

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 10..27

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

AGCACCGGT GAA CAG ACT TTC CAG CTG  
Glu Gln Thr Phe Gln Leu  
1 5

27

10

## (2) INFORMATION FOR SEQ ID NO:66:

15

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

20

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Glu Gln Thr Phe Gln Leu  
1 5

25

## (2) INFORMATION FOR SEQ ID NO:67:

30

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

35

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 11..27

40

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GGAAACCGGT CCG GGA AAG AAA GTG GG  
Pro Gly Lys Lys Val  
1 5

27

50

## (2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

55

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Pro Gly Lys Lys Val  
1 5

60

59

## (2) INFORMATION FOR SEQ ID NO:69:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 208 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

15

Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe  
 1 5 10 15

20

Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp  
 20 25 30

Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys  
 35 40 45

25

Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp  
 50 55 60

30

Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp  
 65 70 75 80

Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His Ala Asn  
 85 90 95

35

Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro Gly Lys  
 100 105 110

Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Pro  
 115 120 125

40

Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly  
 130 135 140

45

Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys  
 145 150 155 160

Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Ile  
 165 170 175

50

Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe  
 180 185 190

Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
 195 200 205

55

## (2) INFORMATION FOR SEQ ID NO:70:

## (i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 208 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

10 Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe  
 1 5 10 15  
 20 Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp  
 20 25 30  
 35 Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys  
 35 40 45  
 50 Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp  
 50 55 60  
 65 Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp  
 65 70 75 80  
 85 Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser Asn  
 85 90 95  
 100 Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly Lys  
 100 105 110  
 115 Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Ser  
 115 120 125  
 130 Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly  
 130 135 140  
 145 Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys  
 145 150 155 160  
 165 Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Met  
 165 170 175  
 180 Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe  
 180 185 190  
 195 Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
 195 200 205

(2) INFORMATION FOR SEQ ID NO:71:

50

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 208 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: protein

60

61

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

5 Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe  
 1 5 10 15  
 Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp  
 20 25 30  
 10 Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys  
 35 40 45  
 Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp  
 50 55 60  
 15 Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp  
 65 70 75 80  
 Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn  
 85 90 95  
 20 Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys  
 100 105 110  
 25 Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro  
 115 120 125  
 Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly  
 130 135 140  
 30 Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys  
 145 150 155 160  
 Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile  
 165 170 175  
 35 Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe  
 180 185 190  
 40 Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
 195 200 205

(2) INFORMATION FOR SEQ ID NO:72:

45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 483 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

55 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..483

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG  
 Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser  
 1 5 10 15

48

62

5	ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys 20 25 30	96
10	CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser 35 40 45	144
15	TTC ACC GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys 50 55 60	192
20	TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp 65 70 75 80	240
25	CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp 85 90 95	288
30	AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly 100 105 110	336
35	ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys 115 120 125	384
40	CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT AGT AAC His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn 130 135 140	432
45	TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT GAG Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu 145 150 155 160	480
50	AAT Asn	483

## (2) INFORMATION FOR SEQ ID NO:73:

45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 161 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
50	(ii) MOLECULE TYPE: protein	
55	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:	
60	Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser 1 5 10 15 Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys 20 25 30 Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser 35 40 45	



[illegible]

25 (2) INFORMATION FOR SEQ ID NO:74:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 705 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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35      (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 1..705

```

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

45	TTG Leu 1	CCC Pro	GCC Ala	CAG Gln	GTG Val 5	GCA Ala	TTT Phe	ACA Thr	CCC Pro	TAC Tyr 10	GCC Ala	CCG Pro	GAG Glu	CCC Pro	GGG Gly 15	AGC Ser	48
50	ACA Thr	TGC Cys	CGG Arg	CTC Leu 20	AGA Arg	GAA Glu	TAC Tyr	TAT Tyr	GAC Asp 25	CAG Gln	ACA Thr	GCT Ala	CAG Gln	ATG Met 30	TGC Cys	TGC Cys	96
55	AGC Ser	AAG Lys	TGC Cys 35	TCG Ser	CCG Pro	GGC Gly	CAA Gln 40	CAT His	GCA Ala	AAA Lys	GTC Val	TTC Phe	TGT Cys 45	ACC Thr	AAG Lys	ACC Thr	144
60	TCG Ser	GAC Asp 50	ACC Thr	GTG Val	TGT Cys	GAC Asp	TCC Ser 55	TGT Cys	GAG Glu	GAC Asp	AGC Ser	ACA Thr 60	TAC Tyr	ACC Thr	CAG Gln	CTC Leu	192
65	TGG Trp 65	AAC Asn	TGG Trp	GTT Val	CCC Pro	GAG Glu 70	TGC Cys	TTG Leu	AGC Ser	TGT Cys	GGC Gly 75	TCC Ser	CGC Arg	TGT Cys	AGC Ser	TCT Ser 80	240

64

	GAC	CAG	GTG	GAA	ACT	CAA	GCC	TGC	ACT	CGG	GAA	CAG	AAC	CGC	ATC	TGC	288
	Asp	Gln	Val	Glu	Thr	Gln	Ala	Cys	Thr	Arg	Glu	Gln	Asn	Arg	Ile	Cys	
					85					90					95		
5	ACC	TGC	AGG	CCC	GGC	TGG	TAC	TGC	GCG	CTG	AGC	AAG	CAG	GAG	GGG	TGC	336
	Thr	Cys	Arg	Pro	Gly	Trp	Tyr	Cys	Ala	Leu	Ser	Lys	Gln	Glu	Gly	Cys	
				100					105					110			
10	CGG	CTG	TGC	GCG	CCG	CTG	CGC	AAG	TGC	CGC	CCG	GGC	TTC	GGC	GTG	GCC	384
	Arg	Leu	Cys	Ala	Pro	Leu	Arg	Lys	Cys	Arg	Pro	Gly	Phe	Gly	Val	Ala	
			115					120					125				
15	AGA	CCA	GGA	ACT	GAA	ACA	TCA	GAC	GTG	GTG	TGC	AAG	CCC	TGT	GCC	CCG	432
	Arg	Pro	Gly	Thr	Glu	Thr	Ser	Asp	Val	Val	Cys	Lys	Pro	Cys	Ala	Pro	
		130					135					140					
20	GGG	ACG	TTC	TCC	AAC	ACG	ACT	TCA	TCC	ACG	GAT	ATT	TGC	AGG	CCC	CAC	480
	Gly	Thr	Phe	Ser	Asn	Thr	Thr	Ser	Ser	Thr	Asp	Ile	Cys	Arg	Pro	His	
	145					150					155					160	
25	CAG	ATC	TGT	AAC	GTG	GTG	GCC	ATC	CCT	GGG	AAT	GCA	AGC	AGG	GAT	GCA	528
	Gln	Ile	Cys	Asn	Val	Val	Ala	Ile	Pro	Gly	Asn	Ala	Ser	Arg	Asp	Ala	
				165						170					175		
30	GTC	TGC	ACG	TCC	ACG	TCC	CCC	ACC	CGG	AGT	ATG	GCC	CCA	GGG	GCA	GTA	576
	Val	Cys	Thr	Ser	Thr	Ser	Pro	Thr	Arg	Ser	Met	Ala	Pro	Gly	Ala	Val	
				180					185					190			
35	CAC	TTA	CCC	CAG	CCA	GTG	TCC	ACA	CGA	TCC	CAA	CAC	ACG	CAG	CCA	ACT	624
	His	Leu	Pro	Gln	Pro	Val	Ser	Thr	Arg	Ser	Gln	His	Thr	Gln	Pro	Thr	
			195					200					205				
40	CCA	GAA	CCC	AGC	ACT	GCT	CCA	AGC	ACC	TCC	TTC	CTG	CTC	CCA	ATG	GGC	672
	Pro	Glu	Pro	Ser	Thr	Ala	Pro	Ser	Thr	Ser	Phe	Leu	Leu	Pro	Met	Gly	
		210					215					220					
45	CCC	AGC	CCC	CCA	GCT	GAA	GGG	AGC	ACT	GGC	GAC						705
	Pro	Ser	Pro	Pro	Ala	Glu	Gly	Ser	Thr	Gly	Asp						
	225					230					235						

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 235 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

55	Leu	Pro	Ala	Gln	Val	Ala	Phe	Thr	Pro	Tyr	Ala	Pro	Glu	Pro	Gly	Ser	
	1				5					10					15		
60	Thr	Cys	Arg	Leu	Arg	Glu	Tyr	Tyr	Asp	Gln	Thr	Ala	Gln	Met	Cys	Cys	
				20					25					30			
	Ser	Lys	Cys	Ser	Pro	Gly	Gln	His	Ala	Lys	Val	Phe	Cys	Thr	Lys	Thr	
			35					40					45				
	Ser	Asp	Thr	Val	Cys	Asp	Ser	Cys	Glu	Asp	Ser	Thr	Tyr	Thr	Gln	Leu	
		50					55					60					

65

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser  
 65 70 75 80  
 5 Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys  
 85 90 95  
 Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys  
 100 105 110  
 10 Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala  
 115 120 125  
 Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro  
 130 135 140  
 Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His  
 145 150 155 160  
 20 Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala  
 165 170 175  
 Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val  
 180 185 190  
 25 His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr  
 195 200 205  
 Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly  
 210 215 220  
 Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp  
 225 230 235

35 (2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 420 amino acids  
 (B) TYPE: amino acid  
 40 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

50 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu  
 1 5 10 15  
 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro  
 20 25 30  
 55 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys  
 35 40 45  
 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys  
 50 55 60  
 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp  
 65 70 75 80

66

	Cys	Arg	Glu	Cys	Glu	Ser	Gly	Ser	Phe	Thr	Ala	Ser	Glu	Asn	His	Leu	
					85					90					95		
5	Arg	His	Cys	Leu	Ser	Cys	Ser	Lys	Cys	Arg	Lys	Glu	Met	Gly	Gln	Val	
				100					105					110			
	Glu	Ile	Ser	Ser	Cys	Thr	Val	Asp	Arg	Asp	Thr	Val	Cys	Gly	Cys	Arg	
10			115					120					125				
	Lys	Asn	Gln	Tyr	Arg	His	Tyr	Trp	Ser	Glu	Asn	Leu	Phe	Gln	Cys	Phe	
		130					135					140					
15	Asn	Cys	Ser	Leu	Cys	Leu	Asn	Gly	Thr	Val	His	Leu	Ser	Cys	Gln	Glu	
		145				150					155					160	
	Lys	Gln	Asn	Thr	Val	Cys	Thr	Cys	His	Ala	Gly	Phe	Phe	Leu	Arg	Glu	
					165					170					175		
20	Asn	Glu	Cys	Val	Ser	Cys	Ser	Asn	Cys	Lys	Lys	Ser	Leu	Glu	Cys	Thr	
				180					185					190			
	Lys	Leu	Cys	Leu	Pro	Gln	Ile	Glu	Asn	Val	Lys	Gly	Thr	Glu	Asp	Ser	
25			195					200					205				
	Gly	Thr	Thr	Gly	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	
		210					215					220					
30	Phe	Phe	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	
		225				230					235					240	
	Val	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	
					245					250					255		
35	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	
				260					265					270			
	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Lys	
40			275					280					285				
	Ile	Ile	Gln	Asp	Ile	Asp	Leu	Cys	Glu	Asn	Ser	Val	Gln	Arg	His	Ile	
		290					295					300					
45	Gly	His	Ala	Asn	Leu	Thr	Phe	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu	Ser	
		305				310					315				320		
	Leu	Pro	Gly	Lys	Lys	Val	Gly	Ala	Glu	Asp	Ile	Glu	Lys	Thr	Ile	Lys	
					325					330					335		
50	Ala	Cys	Lys	Pro	Ser	Asp	Gln	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg	
				340					345					350			
	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	His	Ala	Leu	
55			355					360					365				
	Lys	His	Ser	Lys	Thr	Tyr	His	Phe	Pro	Lys	Thr	Val	Thr	Gln	Ser	Leu	
		370					375					380					
60	Lys	Lys	Thr	Ile	Arg	Phe	Leu	His	Ser	Phe	Thr	Met	Tyr	Lys	Leu	Tyr	
		385				390					395					400	
	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	
					405					410					415		

Ile Ser Cys Leu  
420

5 (2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 211 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

20 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu  
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro  
20 25 30

25 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys  
35 40 45

30 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys  
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp  
65 70 75 80

35 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu  
85 90 95

40 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val  
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg  
115 120 125

45 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe  
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu  
145 150 155 160

50 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu  
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr  
180 185 190

55 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser  
195 200 205

60 Gly Thr Thr  
210

68

## (2) INFORMATION FOR SEQ ID NO:78:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 417 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu  
 1 5 10 15  
 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro  
 20 25 30  
 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys  
 35 40 45  
 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys  
 50 55 60  
 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp  
 65 70 75 80  
 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu  
 85 90 95  
 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val  
 100 105 110  
 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg  
 115 120 125  
 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe  
 130 135 140  
 Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu  
 145 150 155 160  
 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu  
 165 170 175  
 Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr  
 180 185 190  
 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser  
 195 200 205  
 Gly Thr Thr Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg  
 210 215 220  
 Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val  
 225 230 235 240  
 Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile  
 245 250 255

69

Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu  
 260 265 270  
 5 Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln  
 275 280 285  
 Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala  
 290 295 300  
 10 Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly  
 305 310 315 320  
 Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys  
 325 330 335  
 15 Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn  
 340 345 350  
 20 Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser  
 355 360 365  
 Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr  
 370 375 380  
 25 Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu  
 385 390 395 400  
 Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys  
 405 410 415  
 30 Leu

## (2) INFORMATION FOR SEQ ID NO:79:

- 35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 397 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 40 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein

45

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

50 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu  
 1 5 10 15  
 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro  
 20 25 30  
 55 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys  
 35 40 45  
 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys  
 50 55 60  
 60 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp  
 65 70 75 80

70

	Cys	Arg	Glu	Cys	Glu	Ser	Gly	Ser	Phe	Thr	Ala	Ser	Glu	Asn	His	Leu
					85					90					95	
5	Arg	His	Cys	Leu	Ser	Cys	Ser	Lys	Cys	Arg	Lys	Glu	Met	Gly	Gln	Val
				100					105					110		
	Glu	Ile	Ser	Ser	Cys	Thr	Val	Asp	Arg	Asp	Thr	Val	Cys	Gly	Cys	Arg
			115					120					125			
10	Lys	Asn	Gln	Tyr	Arg	His	Tyr	Trp	Ser	Glu	Asn	Leu	Phe	Gln	Cys	Phe
		130					135					140				
	Asn	Cys	Ser	Leu	Cys	Leu	Asn	Gly	Thr	Val	His	Leu	Ser	Cys	Gln	Glu
15		145				150					155					160
	Lys	Gln	Asn	Thr	Val	Cys	Thr	Cys	His	Ala	Gly	Phe	Phe	Leu	Arg	Glu
				165						170					175	
20	Asn	Glu	Cys	Val	Ser	Cys	Ser	Asn	Cys	Lys	Lys	Ser	Leu	Glu	Cys	Thr
				180					185					190		
	Lys	Leu	Cys	Leu	Pro	Gln	Ile	Glu	Asn	Val	Lys	Gly	Thr	Glu	Asp	Ser
			195					200					205			
25	Gly	Thr	Thr	Gly	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp	Asn	Leu	Pro
		210					215					220				
	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His
30		225				230					235					240
	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln
					245					250					255	
35	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu
				260					265					270		
	Cys	Glu	Asn	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr	Phe
			275					280					285			
40	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys	Lys	Val	Gly
		290					295					300				
	Ala	Glu	Asp	Ile	Glu	Lys	Thr	Ile	Lys	Ala	Cys	Lys	Pro	Ser	Asp	Gln
45		305				310					315					320
	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg	Ile	Lys	Asn	Gly	Asp	Gln	Asp
					325					330					335	
50	Thr	Leu	Lys	Gly	Leu	Met	His	Ala	Leu	Lys	His	Ser	Lys	Thr	Tyr	His
				340					345					350		
	Phe	Pro	Lys	Thr	Val	Thr	Gln	Ser	Leu	Lys	Lys	Thr	Ile	Arg	Phe	Leu
			355					360					365			
55	His	Ser	Phe	Thr	Met	Tyr	Lys	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met
		370					375					380				
	Ile	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	Cys	Leu			
60		385				390					395					



71

## (2) INFORMATION FOR SEQ ID NO:80:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 366 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

15

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu  
 1 5 10 15

20

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro  
 20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys  
 35 40 45

25

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys  
 50 55 60

30

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp  
 65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu  
 85 90 95

35

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val  
 100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg  
 115 120 125

40

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe  
 130 135 140

45

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu  
 145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu  
 165 170 175

50

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr  
 180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser  
 195 200 205

55

Gly Thr Thr Gly Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His  
 210 215 220

Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp  
 225 230 235 240

60

Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr  
 245 250 255

72

Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val  
 260 265 270  
 5 Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp  
 275 280 285  
 Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln  
 290 295 300  
 10 Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr  
 305 310 315 320  
 His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe  
 325 330 335  
 15 Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu  
 340 345 350  
 Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
 355 360 365  
 20

## (2) INFORMATION FOR SEQ ID NO:81:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 311 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu  
 1 5 10 15  
 40 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro  
 20 25 30  
 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys  
 35 40 45  
 45 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys  
 50 55 60  
 50 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp  
 65 70 75 80  
 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu  
 85 90 95  
 55 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val  
 100 105 110  
 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg  
 115 120 125  
 60 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe  
 130 135 140

73

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu  
 145 150 155 160  
 5 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu  
 165 170 175  
 Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr  
 180 185 190  
 10 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser  
 195 200 205  
 Gly Thr Thr Gly Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys  
 210 215 220  
 15 Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser  
 225 230 235 240  
 Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met  
 245 250 255  
 20 His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr  
 260 265 270  
 25 Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr  
 275 280 285  
 Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln  
 290 295 300  
 30 Ser Val Lys Ile Ser Cys Leu  
 305 310

## (2) INFORMATION FOR SEQ ID NO:82:

- 35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 40 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein

45

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

50 Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn  
 1 5 10 15  
 Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp  
 20 25 30  
 55 Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly  
 35 40 45  
 Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser  
 50 55 60  
 60 Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val  
 65 70 75 80

Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr  
85 90 95

5 Trp Ser Glu Asn Leu Phe Gln Cys Phe Cys  
100 105

(2) INFORMATION FOR SEQ ID NO:83:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 109 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 15 (ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

	Met 1	Asp	Ser	Val	Cys 5	Pro	Gln	Gly	Lys	Tyr 10	Ile	His	Pro	Gln	Asn 15	Asn
25	Ser	Ile	Cys	Cys 20	Thr	Lys	Cys	His	Lys 25	Gly	Thr	Tyr	Leu	Tyr 30	Asn	Asp
	Cys	Pro	Gly 35	Pro	Gly	Gln	Asp	Thr 40	Asp	Cys	Arg	Glu	Cys 45	Glu	Ser	Gly
30	Ser	Phe 50	Thr	Ala	Ser	Glu	Asn 55	His	Leu	Arg	His	Cys 60	Leu	Ser	Cys	Ser
35	Lys 65	Cys	Arg	Lys	Glu	Met 70	Gly	Gln	Val	Glu	Ile 75	Ser	Ser	Cys	Thr	Val 80
	Asp	Arg	Asp	Thr	Val 85	Cys	Gly	Cys	Arg	Lys 90	Asn	Gln	Tyr	Arg	His 95	Tyr
40	Trp	Ser	Glu	Asn 100	Leu	Phe	Gln	Cys	Phe 105	Asn	Cys	Ser	Leu			

(2) INFORMATION FOR SEQ ID NO:84:

45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 109 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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50      (ii) MOLECULE TYPE: protein

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55 (xi) SEQUENCE DESCRIPTION: SEO ID NO:84:

60 Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn  
1 5 10 15  
Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp  
20 25 30

75

Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly  
 35 40 45  
 5 Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser  
 50 55 60  
 Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val  
 65 70 75 80  
 10 Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr  
 85 90 95  
 Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu  
 100 105  
 15

## (2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 101 amino acids  
 20 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Met Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His  
 1 5 10 15  
 35 Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr  
 20 25 30  
 Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His  
 35 40 45  
 40 Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln  
 50 55 60  
 45 Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys  
 65 70 75 80  
 Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys  
 85 90 95  
 50 Phe Asn Cys Ser Leu  
 100

## (2) INFORMATION FOR SEQ ID NO:86:

55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 91 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

5 Met Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro  
 1 5 10 15  
 Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe  
 20 25 30  
 10 Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys  
 35 40 45  
 Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg  
 50 55 60  
 15 Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser  
 65 70 75 80  
 Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu  
 85 90  
 20

## (2) INFORMATION FOR SEQ ID NO:87:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 94 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

## 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Met Ser Ile Ser Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn  
 1 5 10 15  
 40 Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser  
 20 25 30  
 Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys  
 35 40 45  
 45 Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr  
 50 55 60  
 Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His  
 65 70 75 80  
 Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu  
 85 90  
 50

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising an amino acid  
5 sequence of an osteoprotegerin dimerization domain  
fused to a heterologous amino acid sequence.

2. The polypeptide of Claim 1 wherein the  
heterologous amino acid sequence and the  
10 osteoprotegerin dimerization domain are human.

3. The polypeptide of Claim 1 wherein the  
heterologous amino acid sequence and the  
osteoprotegerin dimerization domain are from different  
15 species.

4. The polypeptide of Claim 1 covalently  
associated with one or more chimeric polypeptides which  
result in a multimeric polypeptide complex.  
20

5. The polypeptide of Claim 4 wherein the complex  
is a dimer.

6. The polypeptide of Claim 1 wherein the  
25 heterologous amino acid sequence is a membrane-bound  
receptor lacking functional membrane associated amino  
acid sequences.

7. The polypeptide of Claim 6 wherein the receptor  
30 is selected from the group consisting of receptor  
tryrosine kinases, cytokine receptors, seven  
transmembrane domain receptors, and cell adhesion  
receptors.

8. The polypeptide of Claim 1 wherein the heterologous amino acid sequence is selected from members of the tumor necrosis factor-like receptor family consisting of TNFR-1, TNFR-2, TNFRp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB.

9. The polypeptide of Claim 8 wherein the heterologous sequence comprises TNFR-1 lacking functional membrane-associated sequences.

10. The polypeptide of Claim 9 wherein the heterologous sequence is a 30 kDa TNF inhibitor, a 40 kDa TNF inhibitor, or an analog thereof.

11. The polypeptide of Claim 1 wherein the carboxy terminus of the heterologous sequence is fused to the amino terminus of the OPG dimerization domain.

12. The polypeptide of Claim 1 wherein the amino terminus of the heterologous sequence is fused to the carboxy terminus of the OPG dimerization domain.

13. The polypeptide of Claim 1 wherein one or more amino acids are inserted between the heterologous sequence and the OPG dimerization domain.

14. A multimeric polypeptide comprising covalently associated monomers of OPG chimeric polypeptides.

15. The multimeric polypeptide of Claim 14 which is a dimer.

16. An isolated nucleic acid sequence encoding the polypeptide of Claim 1.



17. An expression vector comprising the nucleic acid sequence of Claim 16.

5        18. A host cell transformed or transfected with the expression vector of Claim 17 in a manner allowing expression of the nucleic acid.

10       19. A pharmaceutical composition comprising the polypeptide of any of Claims 1 to 15.

FIGURE 1

Rat:	Asn	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	<u>Cys</u>	Glu	Glu	Ala		
Phe Phe														
Mouse:	Lys	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	<u>Cys</u>	Glu	Glu	Ala	Phe	Phe
Human:	Lys	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	<u>Cys</u>	Glu	Glu	Ala	Phe	Phe
Rat:	Arg	Phe	Ala	Val	Pro	Thr	Lys	Ile	Ile	Pro	Asn	Trp	Leu	Ser
Mouse:	Arg	Phe	Ala	Val	Pro	Thr	Lys	Ile	Ile	Pro	Asn	Trp	Leu	Ser
Human:	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser
Rat:	Val	Leu	Val	Asp	Ser	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Mouse:	Val	Leu	Val	Asp	Ser	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Human:	Val	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Rat:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Arg	His	Ser	Ser	Gln	Glu	Gln
Mouse:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Arg	His	Ser	Ser	Gln	Glu	Gln
Human:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln
Rat:	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Arg	Asp
Mouse:	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Arg	Asp
Human:	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp
Rat:	Gln	Glu	Met	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Mouse:	Gln	Glu	Met	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Human:	Gln	Asp	Ile	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Rat:	Glu	Ser	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr
Mouse:	Glu	Ser	Ser	Val	Gln	Arg	His	Leu	Gly	His	Ser	Asn	Leu	Thr
Human:	Glu	Asn	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr
Rat:	Thr	Glu	Gln	Leu	Arg	Ile	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Mouse:	Thr	Glu	Gln	Leu	Leu	Ala	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Human:	Phe	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Rat:	Lys	Ile	Ser	Pro	Asp	Glu	Ile	Glu	Arg	Thr	Arg	Lys	Thr	<u>Cys</u>
Mouse:	Lys	Ile	Ser	Pro	Glu	Glu	Ile	Glu	Arg	Thr	Arg	Lys	Thr	<u>Cys</u>
Human:	Lys	Val	Gly	Ala	Glu	Asp	Ile	Glu	Lys	Thr	Ile	Lys	Ala	<u>Cys</u>
Rat:	Lys	Pro	Ser	Glu	Gln	Leu	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg
Mouse:	Lys	Ser	Ser	Glu	Gln	Leu	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg
Human:	Lys	Pro	Ser	Asp	Gln	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg

FIGURE 1 (Con't)

Rat:	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	Tyr
Mouse:	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	Tyr
Human:	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	His

Rat:	Ala	Leu	Lys	His	Leu	Lys	Ala	Tyr	His	Phe	Pro	Lys	Thr	Val
Mouse:	Ala	Leu	Lys	His	Leu	Lys	Thr	Ser	His	Phe	Pro	Lys	Thr	Val
Human:	Ala	Leu	Lys	His	Ser	Lys	Thr	Tyr	His	Phe	Pro	Lys	Thr	Val

Rat:	Thr	His	Ser	Leu	Arg	Lys	Thr	Ile	Arg	Phe	Leu	His	Ser	Phe
Mouse:	Thr	His	Ser	Leu	Arg	Lys	Thr	Met	Arg	Phe	Leu	His	Ser	Phe
Human:	Thr	Gln	Ser	Leu	Lys	Lys	Thr	Ile	Arg	Phe	Leu	His	Ser	Phe

Rat:	Thr	Met	Tyr	Arg	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile
Mouse:	Thr	Met	Tyr	Arg	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile
Human:	Thr	Met	Tyr	Lys	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile

Rat:	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	<u>Cys</u>	Leu
Mouse:	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	<u>Cys</u>	Leu
Human:	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	<u>Cys</u>	Leu

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FIGURE 2

30kDa TNF Inhibitor

```
5' -GATAGTGTGTGTCCCCAAGGAAAATATATCCACCCTCAAATAATTGCGATTTGCTGTACC-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
D S V C P Q G K Y I H P Q N N S I C C T -  
-AAGTGCCACAAAGGAACCTACTTGTACAATGACTGTCCAGGCCCGGGGCAGGATACGGAC-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
K C H K G T Y L Y N D C P G P G Q D T D -  
-TGCAGGGAGTGTGAGAGCGGCTCCTTCACCGCTTCAGAAAACCACCTCAGACACTGCCTC-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
C R E C E S G S F T A S E N H L R H C L -  
-AGCTGCTCCAAATGCCGAAAGGAAATGGGTCAGGTGGAGATCTCTTCTTGCACAGTGGAC-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
S C S K C R K E M G Q V E I S S C T V D -  
-CGGGACACCGTGTGTGGCTGCAGGAAGAACCAGTACCGGCATTATTGGAGTGAAAACCTT-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
R D T V C G C R K N Q Y R H Y W S E N L -  
-TTCCAGTGCTTCAATTGCAGCCTCTGCCTCAATGGGACCGTGCACCTCTCCTGCCAGGAG-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
F Q C F N C S L C L N G T V H L S C Q E -  
-AAACAGAACACCGTGTGCACCTGCCATGCAGGTTTCTTTCTAAGAGAAAACGAGTGTGTC-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
K Q N T V C T C H A G F F L R E N E C V -  
-TCCTGTAGTAACTGTAAGAAAAGCCTGGAGTGCACGAAGTTGTGCCTACCCCAGATTGAG-  
+-----+-----+-----+-----+-----+-----+-----+-----+  
S C S N C K K S L E C T K L C L P Q I E -  
-AAT-3'  
+-----  
N *
```

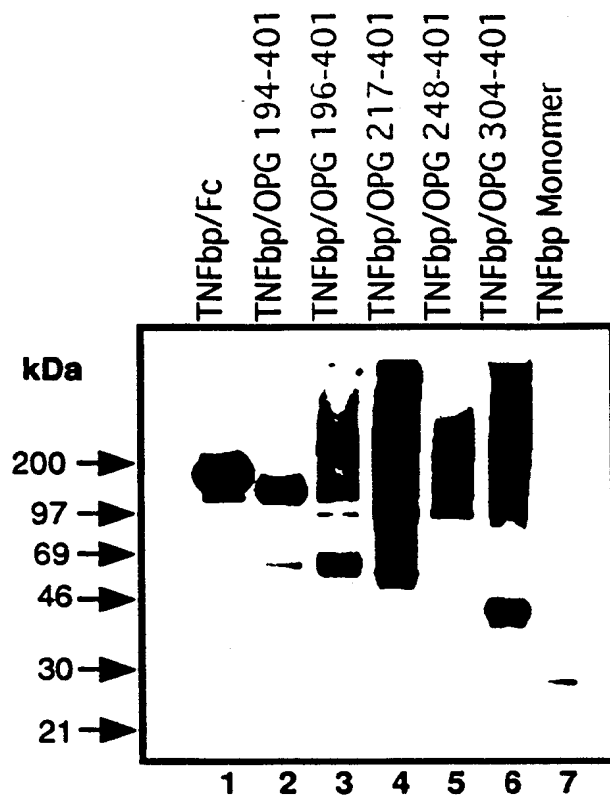


FIGURE 4

	1				50
TNFbp/OPG	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>				DSVCPQGKYI
TNFbp 4.0	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>				DSVCPQGKYI
TNFbp/196	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>				DSVCPQGKYI
TNFbp/217	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>				DSVCPQGKYI
TNFbp/248	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>				DSVCPQGKYI
TNFbp/304	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>				DSVCPQGKYI
	51				100
TNFbp/OPG	HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT				ASENHLRHCL
TNFbp 4.0	HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT				ASENHLRHCL
TNFbp/196	HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT				ASENHLRHCL
TNFbp/217	HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT				ASENHLRHCL
TNFbp/248	HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT				ASENHLRHCL
TNFbp/304	HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT				ASENHLRHCL
	101				150
TNFbp/OPG	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN				FQCFNCSLCL
TNFbp 4.0	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN				FQCFNCSLCL
TNFbp/196	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN				FQCFNCSLCL
TNFbp/217	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN				FQCFNCSLCL
TNFbp/248	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN				FQCFNCSLCL
TNFbp/304	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN				FQCFNCSLCL
	151				200
TNFbp/OPG	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE				CTKLCLPQIE
TNFbp 4.0	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE				CTKLCLPQIE
TNFbp/196	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE				CTKLCLPQIE
TNFbp/217	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE				CTKLCLPQIE
TNFbp/248	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE				CTKLCLPQIE
TNFbp/304	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE				CTKLCLPQIE
	201				250
TNFbp/OPG	NVKGTEDSGT TGKCGIDVTL CEEAFFRFAV PTKFTPNWLS				VLVDNLPQT
TNFbp 4.0	NVKGTEDSGT T.....				.....
TNFbp/196	NVKGTEDSGT T...GIDVTL CEEAFFRFAV PTKFTPNWLS				VLVDNLPQT
TNFbp/217	NVKGTEDSGT TG.....				.....PNWLS VLVDNLPQT
TNFbp/248	NVKGTEDSGT TG.....				.....
TNFbp/304	NVKGTEDSGT TG.....				.....
		196 (OPG)		217 (OPG)	
	251				300
TNFbp/OPG	VNAESVERIK RQHSSQEQT FQLLKLWKHQ				NDQDIVKKII QDIDLCENS
TNFbp 4.0	.....				.....
TNFbp/196	VNAESVERIK RQHSSQEQT FQLLKLWKHQ				NDQDIVKKII QDIDLCENS
TNFbp/217	VNAESVERIK RQHSSQEQT FQLLKLWKHQ				NDQDIVKKII QDIDLCENS
TNFbp/248	.....EQTF FQLLKLWKHQ				NDQDIVKKII QDIDLCENS
TNFbp/304	.....				.....
		248 (OPG)			

	301				350
TNFbp/OPG	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp 4.0	.....	.....	.....	.....	.....
TNFbp/196	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp/217	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp/248	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp/304	.....	.....	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
			304 (OPG)		
	351				400
TNFbp/OPG	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp 4.0	.....	.....	.....	.....	.....
TNFbp/196	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp/217	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp/248	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp/304	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
	401		420		
TNFbp/OPG	QKLFLEMIGN	QVQSVKISCL			
TNFbp 4.0	.....	.....			
TNFbp/196	QKLFLEMIGN	QVQSVKISCL			
TNFbp/217	QKLFLEMIGN	QVQSVKISCL			
TNFbp/248	QKLFLEMIGN	QVQSVKISCL			
TNFbp/304	QKLFLEMIGN	QVQSVKISCL			
			401 (OPG)		

## FIGURE 5

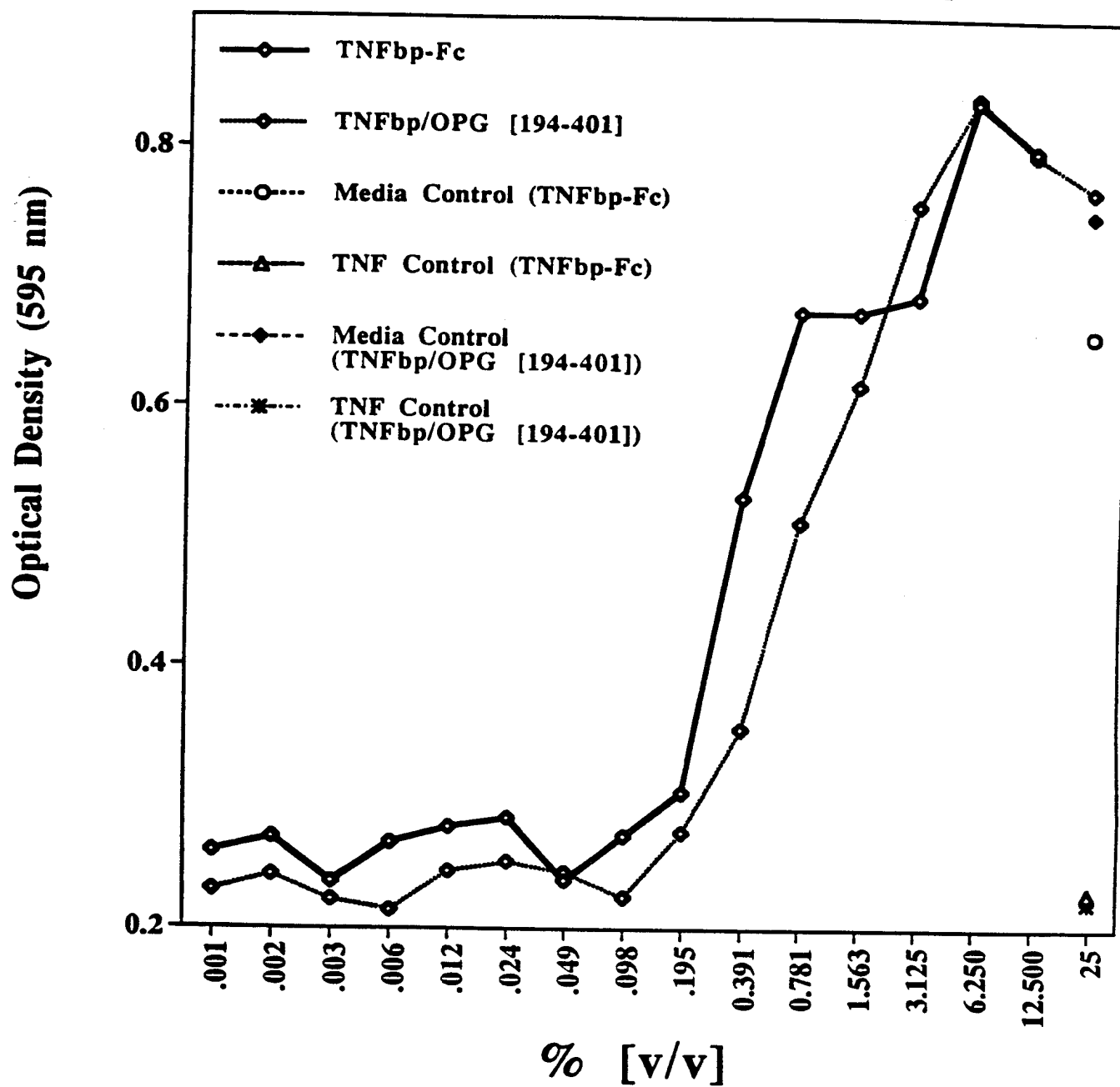




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FIGURE 6

### Inhibition of TNF Cytotoxicity of L929 Murine Connective Tissue Cells



# INTERNATIONAL SEARCH REPORT

Internati Application No

PCT/US 98/08631

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/62 C07K14/705 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	EP 0 816 380 A (SNOW BRAND MILK PROD CO LTD) 7 January 1998 See, in particular, the OCIF-C23S,-CL and -CC mutants in example 22.	1-19
Y	& WO 96 26217 A (SNOW BRAND MILK PRODUCTS) 29 August 1996 ----	1-19
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P,X	WO 97 23614 A (AMGEN INC ;BOYLE WILLIAM J (US); LACEY DAVID L (US); CALZONE FRANK) 3 July 1997 See examples 8.U and 9 ----- -/--	1-3, 11-13, 16-18



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

10 September 1998

Date of mailing of the international search report

25/09/1998

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Lonnoy, 0

# INTERNATIONAL SEARCH REPORT

Internat      Application No

PCT/US 98/08631

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	<p>YAMAGUCHI K ET AL: "Characterisation of structural domains of human osteoclastogenesis inhibitory factor" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 273, no. 9, 27 February 1998, pages 5117-5123, XP002077021</p> <p style="text-align: center;">-----</p>	

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PCT/US 98/08631

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